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Calibration and validation of ultraviolet time-of-flight mass spectrometry for online measurement of exhaled ciprofol

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Ciprofol (HSK 3486, $C_{14}H_{20}O$), a novel 2,6-disubstituted phenol derivative similar to propofol, is a new type of intravenous general anaesthetic. We found that the exhaled ciprofol concentration could be measured online by ultraviolet time-of-flight mass spectrometry (UV-TOFMS), which could be used to predict the plasma concentration and anaesthetic effects of ciprofol. In this study, we present the calibration method and validation results of UV-TOFMS for the quantification of ciprofol gas. Using a self-developed gas generator to prepare different concentrations of ciprofol calibration gas, we found a linear correlation between the concentration and intensity of ciprofol from 0 parts per trillion by level (pptv) to 485.85 pptv ($R^2 = 0.9987$). The limit of quantification was 48.59 pptv and the limit of detection was 7.83 pptv. The imprecision was 12.44% at 97.17 pptv and was 8.96% at 485.85 pptv. The carry-over duration was 120 seconds. In addition, we performed a continuous infusion of ciprofol in beagles, measured the exhaled concentration of ciprofol by UV-TOFMS, determined the plasma concentration by high-performance liquid chromatography, and monitored the anaesthetic effects as reflected by the bispectral index value. The results showed that the exhaled and plasma concentrations of ciprofol were linearly correlated. The exhaled ciprofol concentration correlated well with the anaesthetic effect. The study showed that we could use UV-TOFMS to provide a continuous measurement of gaseous ciprofol concentration at 20 second intervals.

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1 Introduction

Ciprofol (HSK 3486, $C_{14}H_{20}O$), a novel 2,6-disubstituted phenol derivative similar to propofol, has been approved by the National Medical Products Administration of China and has undergone phase III clinical trials in the United States.[1,2] Ciprofol's anaesthetic effects, onset, and recovery time were similar to those of propofol, but the injection pain was significantly reduced.[3–5] The concentration of ciprofol should be timely detected as too high or too low concentrations may increase the incidence of adverse effects including hypotension, bradycardia, respiratory depression, and intraoperative awareness.[5,6]

Like other intravenous anaesthetics, ciprofol is administered primarily on a dose–response basis.[7] Due to differences in drug distribution and metabolism, drug concentrations in the blood and at the site of action are highly variable and difficult to predict from dose and patient characteristics.[8] Overdosing or underdosing of drugs is common in clinical practice. Real-time monitoring of ciprofol for titration to the appropriate dose is essential. However, no studies on real-time monitoring of ciprofol concentration are currently available.

Our team has developed a type of on-line spectrometer that combines ultraviolet (UV) ionization with time-of-flight mass spectrometry (TOFMS) to analyze the concentration of ciprofol in exhaled breath in real time.[9] Calibration is required prior to using this instrument to quantify ciprofol. Therefore, this study was designed to calibrate and analytically validate a UV-TOFMS system for on-line measurement of exhaled ciprofol.

2 Materials and methods

2.1. UV-TOFMS

2.1.1 UV-TOFMS structure. The size of the UV-TOFMS instrument is 520 mm (L) × 550 mm (W) × 940 mm (H), equipped with casters on the bottom for easy pushing. A molecular pump inside is used to maintain a specific level of vacuum, resulting in continuous delivery of air samples under negative pressure (Fig. 1). The pumping speed of the pump to maintain negative pressure inside the chamber is 20 L per

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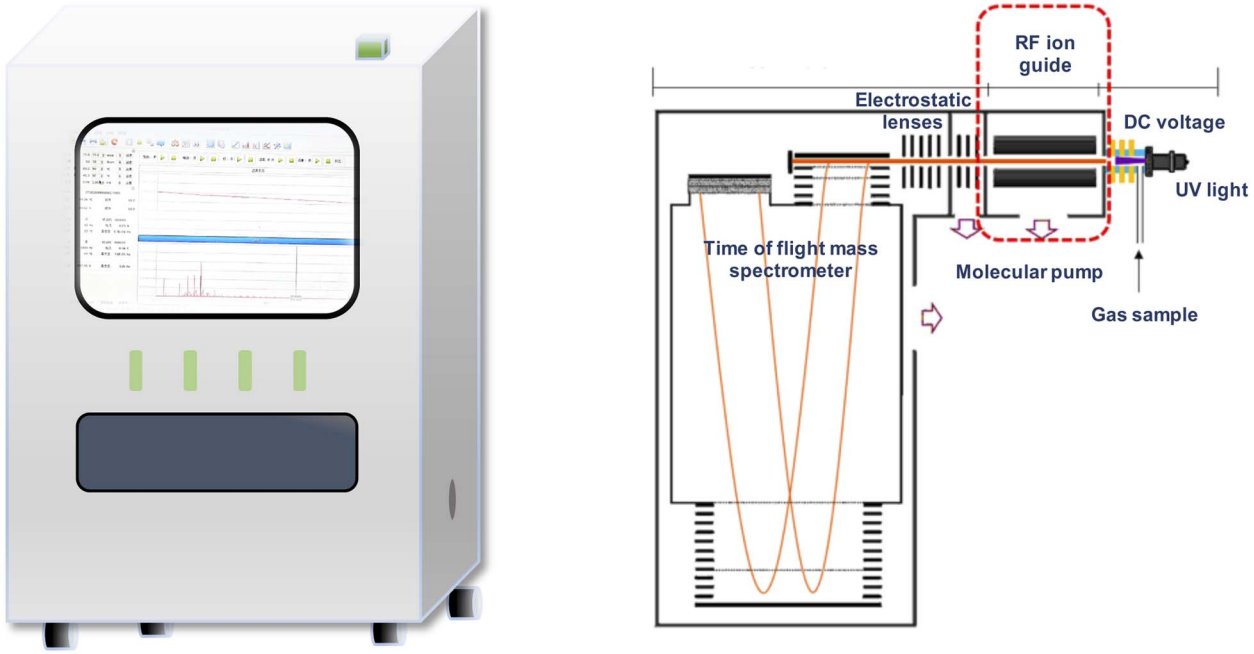

Fig. 1 The interior structure of the UV-TOFMS.

second. The UV light, RF ion guide, electrostatic lenses and TOFMS inside are connected in sequence. A 1.5 m heated (T: 100 °C) polyetherketone (PEEK) tube (OD: 2.5 mm, ID: 2 mm, equipped with a temperature control module and wrapped with sponge, Agilent, California, USA) is used to continuously feed the air samples into the ionization chamber for ionization. To prevent internal pressure buildup, the tubing contains a 1/8″ PEEK tube (Agilent, California, USA) in the centre.

2.1.2 UV-TOFMS workflow. The built-in TOFMS software (version 3.0, Sichuan University, Chengdu, China) allows direct control of parameters such as the carrier gas flow rate, ionization voltage, and acquisition frequency. The operation can be described as follows: the ionization method of the mass spectrometer is phonton ionization which took place in the ionization chamber at a pressure of about 500 Pa. When the UV light is turned on, ions of VOCs in the air samples can be generated by interacting with the photons emitted by UV light. The ionization energy of UV light is 10.6 eV. Inside the ionization chamber, a steady DC voltage applied between two conductive elements gradually decreases from one end to the other, creating a gradient electric field that accelerates the migration of VOCs and sends them into the RF ion guide. The voltage across the ionization chamber is fixed at 25 V. An AC/RF voltage applied to the adjacent conductive rods of the RF ion guide efficiently binds and focuses the ions from the UV photoionization source while separating neutral gas molecules. The ions are then directed to the electrostatic lens, where DC voltages are applied to the conductive components to accelerate, focus, and collimate the ions before they are sent into the TOF-MS. The reflectron type TOF analyzer has a flight tube 265 mm in length. The voltage of the MCP detector is 2400 V. The TOFMS system then measures the time of flight of the ions and determines the mass-to-charge ratio (m/z) and abundance from the velocity differences. The data analysis system can then filter, denoise and analyze the data to identify the amount of ciprofol in the air samples. These data are then displayed on a laptop connected to the instrument (Fig. 2).

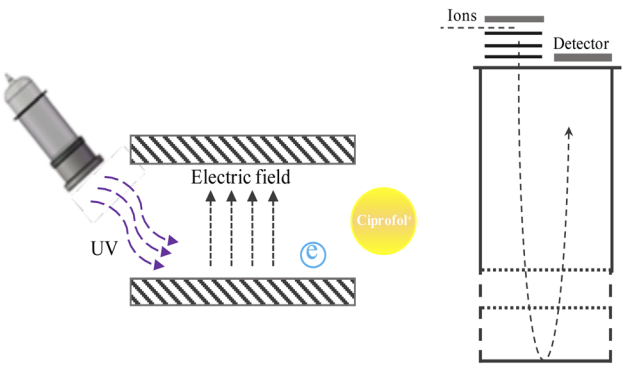

Fig. 2 The workflow of UV-TOFMS.

2.1.3 UV-TOFMS parameters. The ciprofol calibration gas is introduced directly into the UV-TOFMS, with the following detection conditions set for calibration: voltage 77 V; flow rate 70 ml min^{-1}; ion source temperature 100 °C; sample tube temperature 100 °C; sample time 400 000 (*i.e.*, measurement period is 20 seconds per time); pulse interval 50. An m/z of 203.4 in the TOFMS spectrum was selected as the mass peak of ciprofol (*i.e.* the dehydrogenated molecular ion peak), which was obtained by testing ciprofol standards using UV-TOFMS.

2.2. Calibration

2.2.1 Calibration gas generator. The calibration gas generator used in this study is self-constructed (Fig. 3). The

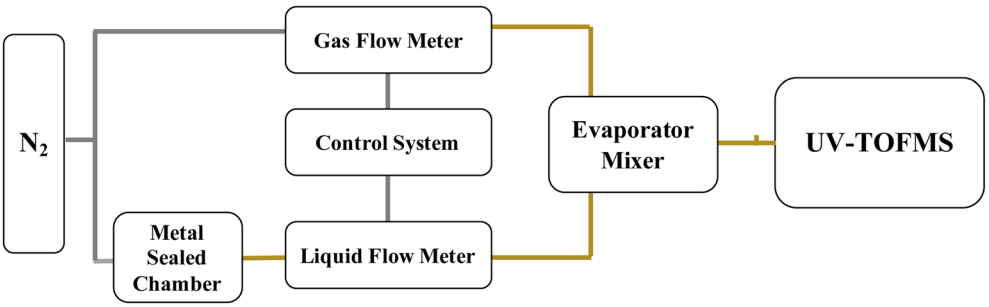

Fig. 3 The composition of the calibration gas generator.

components of the gas generator include a closed metal tank (volume of 60 ml, made of silanized stainless steel), a digital liquid flow meter (μ-FLOW L01, 5 mg h^{-1} to 2 g h^{-1}, Bronkhorst (Shanghai), Shanghai, China), a high-performance mass flow meter for gases (EL-FLOW Select F-111B, 0–16 L h^{-1}, Bronkhorst (Shanghai), Shanghai, China), a controlled evaporator mixer (W-101A, Bronkhorst (Shanghai), Shanghai, China), and a control system (E-8000, Bronkhorst (Shanghai), Shanghai, China). By using the liquid flow meter to accurately control the flow rate of the liquid ciprofol standard and the gas mass flow meter to accurately control the flow rate of carrier gas, liquid ciprofol and the carrier gas enter the controlled evaporator mixer with the set parameters. The concentration of the output diluted standard gas is known from the ratio of the two gases.

2.2.2 Ciprofol stock solutions. Considering the water insolubility of ciprofol, the liquid ciprofol standard was dissolved in methanol first and then diluted with water. Using a 50 μl micro-syringe (Shanghai Gaoge, Shanghai, China), 20 μl of liquid ciprofol standard solution (99% purity, 0.962 g ml^{-1} density, Shimadzu (Shanghai), Shanghai, China) was drawn into a clean glass bottle containing 8 ml of HPLC grade absolute methanol (Shanghai Aladdin, Shanghai, China). Then, the solution was stirred sufficiently and 42 ml of pure water was added to prepare the original stock solution (C_0). According to the formula transformed by using the ideal gas equation $pV = nRT$, the original stock solution after gasification C_0 (parts per billion by volume, ppbv) can be calculated.

$$C_0 = \frac{m_1}{M_1} \times \frac{M_2}{m_2} \times 10^9 \quad (1)$$

In this formula, m_1 is the mass of ciprofol gas (g), M_1 is the molar mass of ciprofol gas (g mol^{-1}), m_2 is the mass of water vapor (g), and M_2 is the molar mass of water vapor (g mol^{-1}). The stock solution was freshly prepared before each experiment. Subsequently, 4 ml of the original stock solution (C_0) was removed and added to 96 ml of pure water to prepare ciprofol solution (C_1).

2.2.3 Diluted ciprofol solutions. According to formula (1), the following concentrations of ciprofol solution after gasification were obtained by mixing the ciprofol solution (C_1) and pure water with a total volume of 50 ml in glass bottles (ciprofol solution (C_1) + pure water): C_2: 0.54 ppbv (0.4 ml + 49.6 ml); C_3: 1.36 ppbv (1 ml + 49 ml); C_4: 2.72 ppbv (2 ml + 48 ml); C_5: 6.79 ppbv (5 ml + 45 ml); C_6: 13.58 ppbv (10 ml + 40 ml). 1000 μl and 10 ml syringes were used for dosing into the glass bottles.

2.2.4 Calibration gas. The above concentration of ciprofol solution was placed in a pressure-tight metal chamber connected to the liquid flow meter and the connected PEEK tube was pre-filled with ciprofol solution. The actual concentration of diluted ciprofol calibration gas (C_i) was calculated using the following formula (2).

$$C_i = \frac{V_{ciprofol}}{(V_{ciprofol} + V_{N_2})} \times C_1 \quad (2)$$

By replacing the ciprofol solution (C_2–C_6, flow rate 0.8 g h^{-1}) and changing the carrier gas flow rate (N_2, flow rate 600 ml h^{-1}, 800 ml h^{-1}), different concentrations of ciprofol calibration gas (0 parts per trillion by level (pptv), 15.66 pptv, 19.43 pptv, 39.15 pptv, 48.59 pptv, 78.30 pptv, 97.17 pptv, 195.74 pptv, 242.93 pptv, 391.48 pptv, and 485.85 pptv) can be obtained from the metal evaporator (150 °C). Then the ciprofol calibration gas is directly introduced into the UV-TOFMS. Each concentration was held for 10 min, corresponding to 30 measurements. Before and after each concentration, 10 blank measurements were obtained.

2.3. Validation

2.3.1 Linearity. The measured UV-TOFMS signal intensity of ciprofol was plotted against the gas concentration of ciprofol from the in-house developed gas generator, excluding the 5 initial and final measurements at each concentration. Linear regression was used to determine the slope, intercept, and linear range.

2.3.2 Limit of detection/limit of quantification. The limit of detection (LOD) and limit of quantification (LOQ) were determined in accordance with the International Conference on Harmonization of "Technical Requirements for Registration of Pharmaceuticals for Human Use".[10] The signal-to-noise ratio was 3 : 1 for the LOD and 10 : 1 for the LOQ. Specifically, the test concentration was gradually reduced by 0.1 ml from the ciprofol concentration (C_1) solution (*i.e.*, 0.3 ml + 49.7 ml, 0.2 ml + 49.8 ml, 0.1 ml + 49.9 ml). Each concentration was maintained for 10 minutes. The noise was calculated as the average intensity of 10 initial blank measurements.

2.3.3 Precision. Method precision was analyzed by holding concentrations of 97.17 pptv and 485.85 pptv with the reference generator for 1 hour while measuring continuously with UV-TOFMS. The standard deviation was determined and imprecision was also estimated as the difference between the highest and lowest readings as a percentage of the average.

2.3.4 Carry over. Concentrations of 97.17 pptv and 485.85 pptv were maintained for 1 hour before changing the concentration to 0 pptv. Concentrations were monitored for the next 15 minutes to determine the time required for the ciprofol signal to disappear.

2.4. Animal experiments

This study was approved by the Animal Ethics Committee of West China Hospital, Sichuan University (20220420001). Five male beagles (12–18 months, 9–14 kg, Chengdu DOSSY Experimental Animals Co., Ltd.) were involved in this study. Bilateral forearm veins were punctured and catheterized (one side for drug administration and the other for blood sampling). The interval of blood sampling was 10 min. Then, the bispectral index (BIS, an indicator of the anaesthetic effect) electrodes were then placed on the beagles' foreheads to monitor the depth of anaesthesia, and the data acquisition interval was 20 seconds. The anaesthetic tiletamine hydrochloride and zolazepam hydrochloride for injection (Zoletil® 50, Virbac Group, France) was injected intravenously at a dose of 5 mg kg^{-1} for anaesthesia induction. After the loss of the righting reflex, a tracheal tube (ID: #7.0; made of polyvinyl chloride; Tuoren, Zhengzhou, China) was intubated under a laryngoscope and connected to a ventilator (Boaray 700, Probe Company, Shenzhen, China), the parameters of which were set as follows: volume control ventilation mode, pure oxygen supply, a flow rate of 2 L min^{-1}, a respiratory rate of 20 times per min, and a tidal volume of 10 ml kg^{-1}. The dosage of ciprofol administered was 0.125 mg kg^{-1} min^{-1} (dose that eliminated the righting reflex in 95% of beagles) for up to 1 hour (BeneFusion n series, Shenzhen Mindray, Shenzhen, China). During the whole procedure, the body temperature of beagles was maintained at 36.5 ± 0.5 °C. Upon awakening, the tracheal tube and the intravenous needle cannula were removed. After full recovery, the beagles were returned to the animal room.

A three-way valve (made of polycarbonate) was used to connect the tracheal tube of the breathing circuit (made of polytetrafluoroethylene) to the UV-TOFMS. Detection conditions were set as shown above. Ciprofol was continuously detected at 20 seconds per time. An *m*/*z* of 203.4 in the TOFMS spectrum was selected as the mass peak of ciprofol. The ciprofol concentration in exhaled air was calculated according to the calibration curve.

2.5. Statistical analysis

Data analysis for the TOFMS spectra of ciprofol and peak intensities was performed by the built-in calculation software. Statistical analyses were performed with Graphpad Prism (version 9.5, Graphpad Prism Software, USA). The Shapiro–Wilk test was used to assess the normality of data. One-way ANOVA was used for normally distributed data. Alternatively, the one-way ANOVA on rank was conducted. $P < 0.05$ was considered significant.

3 Results

3.1. The linear calibration curves

Fig. 4 shows the calibration curve of ciprofol signal intensity against gas concentration in UV-TOFMS. The middle 20 (of 30) values of each concentration were evaluated (gray dots). The linear calibration curve (blue line) shows a coefficient of determination of 0.9987 ($y = 43.02x + 210.0$) between 0 pptv and 485.85 pptv.

3.2. LOD and LOQ

Based on the signal-to-noise ratio, the LOD was 7.83 pptv (average peak intensity: 668) and the LOQ was 48.59 pptv (average peak intensity: 2056).

3.3. Precision

The precision measurements at 97.17 pptv and 485.85 pptv had a relative standard deviation of 6.53% and 4.40% and a statistical range of 12.44% and 8.96% of the mean.

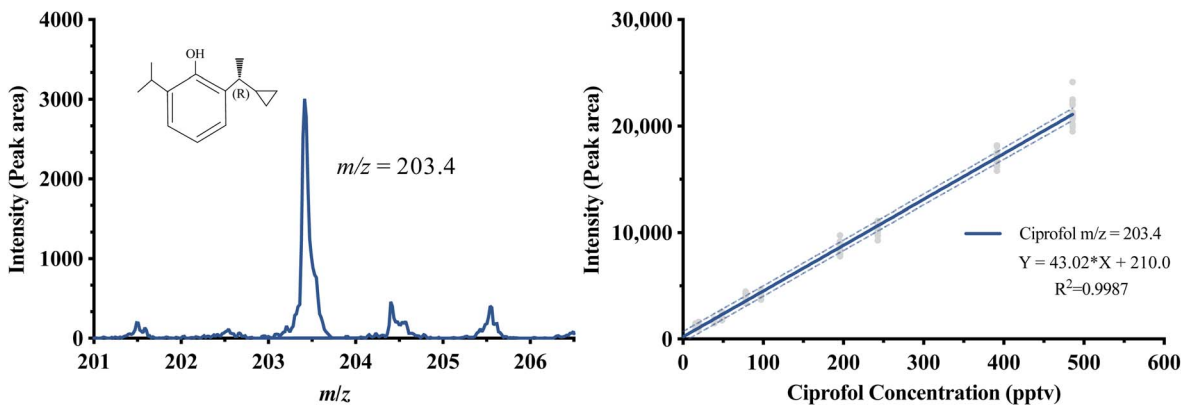

Fig. 4 The *m*/*z* of ciprofol in TOFMS spectra and the calibration curve.

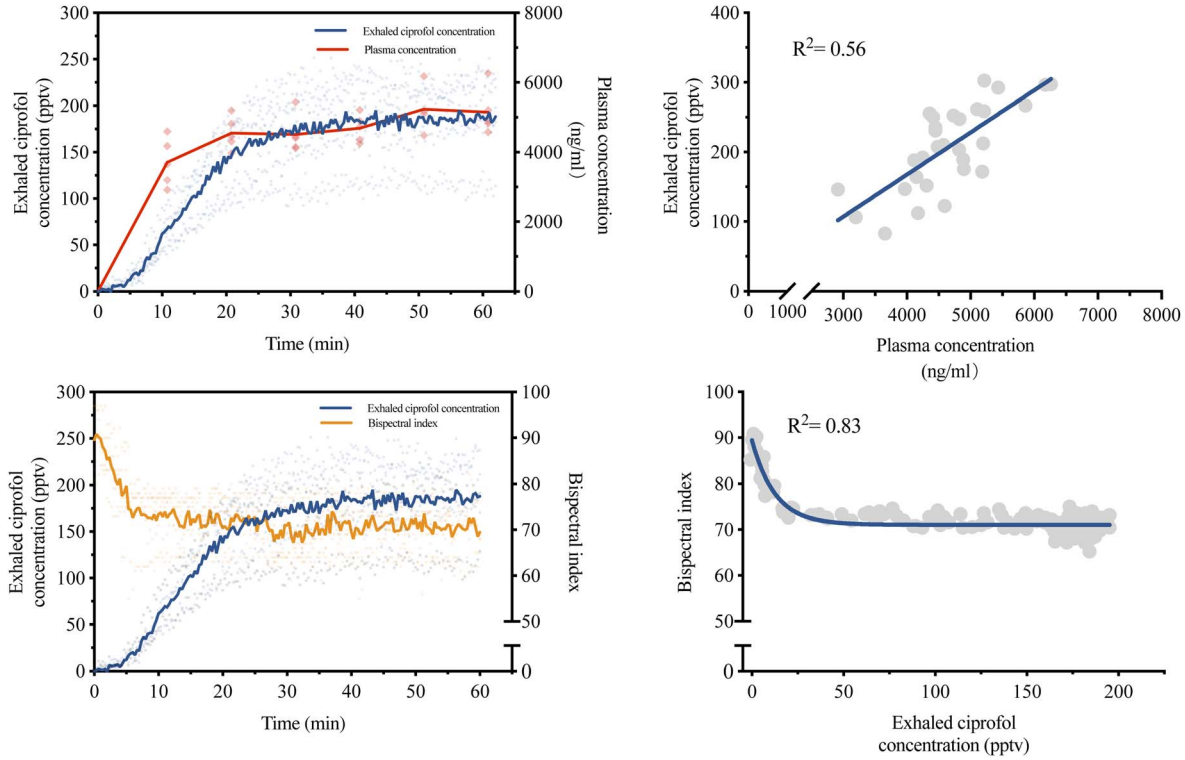

Fig. 5 The correlations of exhaled ciprofol concentration and plasma concentration and BIS. Notes: ciprofol concentrations in exhaled breath and plasma were linearly correlated ($y = 0.06058x - 74.75$, $R^2 = 0.56$); the correlation of exhaled ciprofol concentration and BIS can be well fitted with the one-phase exponential decay model ($y = 18.39\exp(-0.07888x) + 89.44$, $R^2 = 0.83$).

3.4. Carry over

Concentrations of 97.17 pptv and 485.85 pptv were maintained for 1 h before changing the concentration to 0 pptv. The first value after the ciprofol evaporation stopped shows a carry-over of 3.2% and 5.9%, respectively, of the average signal intensity at 97.17 pptv and 485.85 pptv.

3.5. Animal experiments

A total of 5 beagles were involved in this experiment, with an age of 12.89 ± 1.55 month and weight of 11.83 ± 1.13 kg. All beagles survived and were returned to the animal room after full awakening. The administration dosage was 91.50 ± 1.62 mg. The results showed that ciprofol concentrations in exhaled breath and plasma were linearly correlated ($y = 0.06058x - 74.75$, $R^2 = 0.56$), and the correlation of exhaled ciprofol concentration and BIS can be well fitted with the one-phase exponential decay model ($y = 18.39\exp(-0.07888x) + 89.44$, $R^2 = 0.83$) (Fig. 5).

4 Discussion

Online monitoring of the concentrations of anaesthetics is a major problem in clinical anaesthesia and one of the important directions for future anaesthesia research. The only intravenous anaesthetic that can be monitored online in published studies is propofol. Our study is the first to achieve online monitoring of anaesthetics other than propofol.

Current techniques for on-line monitoring of propofol include proton transfer reaction mass spectrometry (PTR-MS),[11] selected ion flow tube mass spectrometry (SIFT-MS),[12] ion molecular reaction mass spectrometry (IMR-MS),[13] gas chromatography combined with a surface acoustic wave sensor (GC-SAW),[14] and ion mobility spectrometry (IMS).[15] The first three are reported to be not suitable in the operating room due to their large instrument size, loud operating noise and complex operation.[16] In studies using GC-SAW, exhaled breath was collected offline, which may have resulted in the loss of important drug concentration information.[17] The performance of IMS is easily disturbed by humidity, which is high in exhaled breath.[18] Using UV-TOFMS, gas samples can be analysed online without pre-separation. The sensitivity of UV-TOFMS can reach the pptv level and it is insensitive to humidity, which meets the requirements for the detection of trace volatile organic compounds in exhaled breath.

In the pilot trials, we have detected the presence of ciprofol in exhaled breath of rats and beagles using the UV-TOFMS instrument and found that the levels of ciprofol in exhaled breath were under 400 pptv. Therefore, to validate this calibration method, we prepared the target standard gas concentration in the range of 0 pptv to 485.85 pptv in this study. The range of ciprofol concentrations in human exhaled breath is unknown because we have not yet used the device on humans to measure ciprofol concentrations. However, ciprofol is a reconstituted drug of propofol with relatively similar

physicochemical properties.[3] Combined with the distribution of data from our completed UV-TOFMS-based studies on propofol in rats, beagles, and humans, we believe that the range of ciprofol concentrations in beagle exhaled breath at equivalent doses is similar to that in humans. Therefore, the concentration ranges investigated in this study are suitable for future extension to humans. The reason why the concentration of ciprofol in expired air is so low, in our opinion, is related to the saturated vapor pressure of ciprofol.[16] The volatility of a substance is related to its saturated vapor pressure, which is lower for propofol.[19] Like propofol, ciprofol is more lipid soluble, and less volatile gas is formed from free ciprofol in the blood and passes through the alveolar membrane.[20] Our study is the first to report the presence of ciprofol in exhaled breath. There are no other studies available for comparison.

The low concentrations of ciprofol can be well measured using UV-TOFMS, which is a mass spectrometer based on UV recombination ionization and time-of-flight mass spectrometry technology. With the soft ionization of UV, pre-separation of air samples is unnecessary, and most volatile organic compounds in air samples can be ionized immediately. Unlike electron ionization, UV ionization can avoid the production of fragment ions, allowing for the direct detection of prototypes.[21] In addition, about 23 ml of air sample is directly delivered into the UV-TOFMS under constant pressure without any other processing. This instrument did not extract more gas compared to other instruments.[22] In addition, the instrument adopts multi-stage vacuum with high-efficiency ion transfer design, which greatly improves the performance of the instrument. When combined with TOFMS, this instrument has the capability for performing online analysis with high sensitivity up to the pptv level. The response time of the instrument is less than 0.5 seconds. Volatile organic compounds in air samples can be ionized instantaneously and simultaneously, and the results of all component concentrations can be calculated in real time by the built-in software, allowing dynamic data acquisition. However, it should be mentioned that the size of the current prototype device is not too small, and requires a certain space in the operating room. The internal vacuum molecular pump takes up a larger space. In the future, the size of the pump can be reduced accordingly. This will reduce the volume of the entire machine.

Ciprofol is modified from propofol, so it has similar physical and chemical properties to those of propofol, *i.e.* it also has high viscosity, which places higher demands on the tubing material in the calibration system.[21] We used an inert material PEEK tube for the introduction of ciprofol into the instrument and wrapped it with sponge. The temperature was controlled at 100 °C by using a temperature control switch. The temperature-controlled sampling tube can effectively avoid the residue of ciprofol gas.[23] It can also speed up the response process of the instrument. In the calibration system, PEEK tubing or an inert metal cavity was also selected as the connection for the piping through which ciprofol passed. This was done to minimize the loss caused by the transfer process.

Unlike previous studies, we did not use commercial off-the-shelf gas generators to produce standard gas.[24] This is because the finished gas generator often has a certain volume. It is difficult to reprocess and fuse it into the instrument. We hope that when the UV-TOFMS instrument is used for clinical testing in the future, the calibration system can be equipped inside the instrument, and the mass flow meter based on the scattering can be freely assembled. Therefore, we built a gas generator ourselves using two precision mass flow meters. The calibration and validation of UV-TOFMS is based on measurements of defined ciprofol gas concentrations. In this method, the gas generator can prepare the target standard gas concentration by changing the initial solution concentration of ciprofol and the carrier gas in combination with the ideal gas equation. The advantage is that the preparation method is simple and convenient, and is also more accurate than the gas dilution method using Tedlar bags.[17] The disadvantage is that the generator uses the ideal gas law to estimate the concentration, which is a general method but not completely accurate.[25] However, based on previous studies, it can be assumed that the associated bias is negligible. Meanwhile, the concentration of integer multiples cannot be obtained. In addition, we chose to set the evaporation tank temperature at 150 °C. The gasification temperature of propofol reported in previous studies was 40 °C or 100 °C, which leaves the possibility of incomplete gasification.[24,26–28] Therefore, in this study, the temperature in the evaporation tank was set at 150 °C to completely gasify the ciprofol solution.

A LOD of 7.83 pptv and a LOQ of 48.59 pptv were obtained with a goodness of fit of 0.9987. This linearity range is well suited for calibration of ciprofol gas concentrations in rats and beagles, and sufficient to quantify ciprofol over the entire clinical range. We used as many as 11 points instead of the traditional 5 points when drawing the calibration curve. We hoped that adding more points would make the standard curves more accurate, since this was an exploratory study. Based on the exploration of good linearity, we plan to reduce the number of calibration points in the next step. Our ultimate goal is to reduce the cost and time required for gas calibration by integrating the calibration system into the UV-TOFMS and controlling the variation of the carrier gas flow rate only, without changing the concentration of the ciprofol solution. Within a good linear concentration range, it is possible to produce standard gases with different concentrations at two or three points, which would make calibration of the system easy and quick. Regarding accuracy, our data shows low variation, less than 15%. A variation of up to 15% is generally considered satisfactory from an analytical point of view.[22] In this system, we used constant pressure injection to ensure the measurement accuracy of the instrument in different detection periods to a certain extent. However, the effect of 12.44% breath inaccuracy on blood concentration estimation is unclear. A carry-over effect was observed in the first 20 s after a concentration change, with a value of less than 6% of the average signal intensity for 97.17 pptv and 485.85 pptv. One reason for this effect may be the adhesion of ciprofol to the internal surfaces of the gas generator and the UV-TOFMS.[29–31] All of these surfaces have the potential to contribute to carry over. The interaction of gaseous ciprofol with plastic and stainless-steel surfaces should

be further investigated. However, after six measurements at 20 s intervals, the carry-over was only 2.2% at 0 pptv, which is less than the imprecision of our method. Furthermore, the low carry-over after only one minute of washout is suitable for clinical use.

From data on continuous infusion of ciprofol in beagles, we found a moderate correlation between the exhaled concentration and the plasma concentration. This may be due to the small number of subjects. However, as an exploratory experiment, this suggests that the exhaled concentration of ciprofol has the potential to predict the plasma concentration. However, as with propofol, there appears to be a significant delay in the exhaled concentration of ciprofol compared to the plasma concentration.[19,31] In fact, when the corresponding standard gas in the gas generator is connected to the UV-TOFMS, the instrument can detect the presence of ciprofol in the next moment, indicating that these delays are not caused by the adsorption of the tubing, but rather by the enormous blood gas distribution coefficient of ciprofol. Achieving equilibrium between the free ciprofol in the blood and the volatile ciprofol gas takes a long time. This delay can be also exacerbated by blockage of the alveolar membrane.[32] From another perspective, there is a certain delay between plasma drug concentrations and anaesthetic effects.[33–35] Therefore, we further investigated whether exhaled ciprofol concentrations correlated well with anaesthetic effects. We found that the relationship between exhaled ciprofol concentration and BIS could be well fitted by the one-phase exponential decay model ($R^2 = 0.83$), indicating that exhaled ciprofol monitoring can be used to guide the titration of depth of anaesthesia, which is more helpful to anaesthetists.

In this study, we developed and validated a method based on UV-TOFMS for the quantitative detection of ciprofol gas. In fact, this method including the UV-TOFMS instrument, the gas generator, and the gas dilution method is not only suitable for ciprofol, but also for propofol. It is inferred that propofol and ciprofol have similar structures and physicochemical properties. We have completed a series of experiments using the same calibration method for the quantitative detection of propofol gas and also obtained good results. Meanwhile, we are actively investigating the feasibility of this calibration method for other anaesthetics, such as etomidate, another liposoluble anaesthetic commonly used in clinical practice. Of course, this method also has some limitations. Firstly, the humidity of the standard gases obtained by this method is around 100%. However, the amount of water in exhaled breath is not constant during the whole surgery and the humidity may change over time. Therefore, this method needs to be further improved in future studies to achieve the purpose of obtaining gases with different humidities. Secondly, in clinical practice, anaesthesia is often the result of a combination of different anaesthetic agents, including sedatives (*e.g.* ciprofol and propofol), analgesics and muscle relaxants, rather than a single drug. Whether the calibration system and method can be used for the simultaneous quantitative detection of other intravenous anaesthetics requires further investigation.

5 Conclusions

The calibration and validation procedure of a UV-TOFMS with a self-developed gas generator was successfully established. The results showed that we could use the UV-TOFMS to provide continuous measurement of gaseous ciprofol concentration at 20 second intervals.

Author contributions

Funding acquisition and project administration: ZWS; supervision: DYX; conceptualization: ZWS, LXX, and CP; methodology and resources: ZZJ, LWW, and DYX; investigation and validation: LXX, CP, and LX; data curation and software: LWW and LXX; formal analysis: LXX, CP, and KY; visualization and writing – original draft: LXX and CP; writing – review & editing: ZWS, LXX, CP, ZZJ, and DYX. All authors approved the submitted version of the manuscript and agreed to be personally accountable for their own contributions and to ensure that questions related to the accuracy and integrity of the work are resolved and documented.

Conflicts of interest

The authors declare no conflict of interest for this work.

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References

1 *NCT05478174, Efficacy and Safety of HSK3486 Compared to Propofol for Adults Undergoing Elective Surgery with General Anaesthesia*, **https://www.clinicaltrials.gov/**, 2023-02-22.
2 P. Liang, M. Dai, X. Wang, *et al.*, Efficacy and safety of ciprofol *vs.* propofol for the induction and maintenance of general anaesthesia: a multicentre, single-blind, randomised, parallel-group, phase 3 clinical trial, *Eur. J. Anaesthesiol.*, 2023, **40**, 399–406.
3 J. Liao, M. Li, C. Huang, *et al.*, Pharmacodynamics and Pharmacokinetics of HSK3486, a Novel 2,6-Disubstituted Phenol Derivative as a General Anaesthetic, *Front. Pharmacol.*, 2022, **13**, 830791.
4 Y. Teng, M. Ou, X. Wang, *et al.*, Efficacy and safety of ciprofol for the sedation/anaesthesia in patients undergoing colonoscopy: phase IIa and IIb multi-center clinical trials, *Eur. J. Pharm. Sci.*, 2021, **164**, 105904.
5 Z. Luo, H. Tu, X. Zhang, *et al.*, Efficacy and Safety of HSK3486 for Anaesthesia/Sedation in Patients Undergoing Fiberoptic Bronchoscopy: A Multicenter, Double-Blind, Propofol-Controlled, Randomized, Phase 3 Study, *CNS Drugs*, 2022, **36**, 301–313.

6 J. Li, X. Wang, J. Liu, *et al.*, Comparison of ciprofol (HSK3486) versus propofol for the induction of deep sedation during gastroscopy and colonoscopy procedures: a multi-centre, non-inferiority, randomized, controlled phase 3 clinical trial, *Basic Clin. Pharmacol. Toxicol.*, 2022, **131**, 138–148.

7 B. Meibohm and H. Derendorf, Basic concepts of pharmacokinetic/pharmacodynamic (PK/PD) modelling, *Int. J. Clin. Pharmacol. Ther.*, 1997, **35**, 401–413.

8 M. M. Struys, M. Sahinovic, B. J. Lichtenbelt, *et al.*, Optimizing intravenous drug administration by applying pharmacokinetic/pharmacodynamic concepts, *Br. J. Anaesth.*, 2011, **107**, 38–47.

9 Y. X. Duan, Z. J. Zhao and Y. T. Yang, A high-sensitivity UV-ionization time-of-flight mass spectrometer and ion time-of-flight measurement method, *China Pat.*, CN111613514A, 2020.09.01.

10 *ICH Topic Q2 (R1) Validation of Analytical Procedures: Text and Methodology*, Int. Conf. Harmon., 2005, **1994**, p. 17.

11 G. R. Harrison, A. D. Critchley, C. A. Mayhew and J. M. Thompson, Real-time breath monitoring of propofol and its volatile metabolites during surgery using a novel mass spectrometric technique: a feasibility study, *Br. J. Anaesth.*, 2003, **91**, 797–799.

12 P. R. Boshier, J. R. Cushnir, V. Mistry, *et al.*, On-line, real time monitoring of exhaled trace gases by SIFT-MS in the perioperative setting: a feasibility study, *Analyst*, 2011, **136**, 3233–3237.

13 M. Grossherr, B. Varadarajan, L. Dibbelt, *et al.*, Time course of ethanol and propofol exhalation after bolus injection using ion molecule reaction-mass spectrometry, *Anal. Bioanal. Chem.*, 2011, **401**, 2063–2067.

14 H. Dong, F. Zhang, J. Chen, *et al.*, Evaluating Propofol Concentration in Blood From Exhaled Gas Using a Breathing-Related Partition Coefficient, *Anesth. Analg.*, 2020, **130**, 958–966.

15 Y. Liu, Y. Gong, C. Wang, *et al.*, Online breath analysis of propofol during anesthesia: clinical application of membrane inlet-ion mobility spectrometry, *Acta Anaesthesiol. Scand.*, 2015, **59**, 319–328.

16 L. M. Wirtz, S. Kreuer, T. Volk and T. Hüppe, Modern breath analysis, *Med. Klin. - Intensivmed. Notfallmedizin*, 2019, **114**, 655–660.

17 X. Chen, X. L. Zhang, L. Liu, *et al.*, Gas chromatograph-surface acoustic wave for quick real-time assessment of blood/exhaled gas ratio of propofol in humans, *Br. J. Anaesth.*, 2014, **113**, 807–814.

18 T. Teucke, F. Maurer, L. M. Müller-Wirtz, T. Volk, D. I. Sessler and S. Kreuer, Humidity and measurement of volatile propofol using MCC-IMS (EDMON), *J. Clin. Monit. Comput.*, 2023, **37**, 493–500.

19 C. Hornuss, S. Praun, J. Villinger, *et al.*, Real-time monitoring of propofol in expired air in humans undergoing total intravenous anaesthesia, *Anesthesiology*, 2007, **106**, 665–674.

20 S. Kiem and J. J. Schentag, Interpretation of antibiotic concentration ratios measured in epithelial lining fluid, *Antimicrob. Agents Chemother.*, 2008, **52**, 24–36.

21 Z. J. Zhao, F. Y. He, J. Chen, *et al.*, Development of Membrane Inlet Based on Ultra Violet Ionization Time of Flight Mass Spectrometer and Its Application in VOCs Analysis at Workplace, *J. Chinese Mass Spectrom. Soc.*, 2021, **42**, 16.

22 F. Maurer, L. Walter, M. Geiger, *et al.*, Calibration and validation of a MCC/IMS prototype for exhaled propofol online measurement, *J. Pharm. Biomed. Anal.*, 2017, **145**, 293–297.

23 G. Skirbutis, A. Dzingutė, V. Masiliūnaitė, *et al.*, PEEK polymer's properties and its use in prosthodontics. A review, *Stomatologija*, 2018, **20**, 54–58.

24 F. Maurer, M. Geiger, T. Volk, D. I. Sessler and S. Kreuer, Validation of liquid and gaseous calibration techniques for quantification of propofol in breath with sorbent tube thermal desorption system GC-MS, *J. Pharm. Biomed. Anal.*, 2017, **143**, 116–122.

25 D. M. Avishay and K. M. Tenny, *Henry's Law*, StatPearls Publishing, Treasure Island (FL), 2022.

26 S. Kamysek, P. Fuchs, H. Schwoebel, J. P. Roesner, S. Kischkel, K. Wolter, C. Loeseken, J. K. Schubert and W. Miekisch, Drug detection in breath: effects of pulmonary blood flow and cardiac output on propofol exhalation, *Anal. Bioanal. Chem.*, 2011, **401**, 2093–2102.

27 W. Miekisch, P. Fuchs, S. Kamysek, C. Neumann and J. K. Schubert, Assessment of propofol concentrations in human breath and blood by means of HS-SPME-GC–MS, *Clin. Chim. Acta*, 2008, **395**, 32–37.

28 T. Birken, J. Schubert, W. Miekisch and G. Nöldge-Schomburg, A novel visually CO2 controlled alveolar breath sampling technique, *Technol. Health Care*, 2006, **14**, 499–506.

29 D. Lorenz, F. Maurer, K. Trautner, *et al.*, Adhesion of volatile propofol to breathing circuit tubing, *J. Breath Res.*, 2017, **11**, 036005.

30 F. Maurer, D. J. Lorenz, G. Pielsticker, *et al.*, Adherence of volatile propofol to various types of plastic tubing, *J. Breath Res.*, 2017, **11**, 016009.

31 J. W. Sall and J. Leong, Technical communication: stability of propofol in polystyrene-based tissue culture plates, *Anesth. Analg.*, 2013, **117**, 65–67.

32 C. Berchtold, M. Bosilkovska, Y. Daali, B. Walder and R. Zenobi, Real-time monitoring of exhaled drugs by mass spectrometry, *Mass Spectrom. Rev.*, 2014, **33**, 394–413.

33 N. H. G. Holford and L. B. Sheiner, Understanding the dose-effect relationship, *Clin. Pharmacokinet.*, 1981, **6**, 429–445.

34 A. Misra, S. Ganesh, A. Shahiwala and S. P. Shah, Drug delivery to the central nervous system: a review, *J. Pharm. Pharm. Sci.*, 2003, **6**, 252–273.

35 C. Hornuss, D. Wiepcke, S. Praun, *et al.*, Time course of expiratory propofol after bolus injection as measured by ion molecule reaction mass spectrometry, *Anal. Bioanal. Chem.*, 2012, **403**, 555–561.

