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1,2-Dimethylimidazole-4-sulfonyl chloride (DMISC) – a novel derivatization strategy for the analysis of propofol by LC-ESI-MS/MS

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Aim: Analysis of the anaesthetic agent propofol in biological samples by LC-MS/MS is a great challenge due to weak fragmentation and poor ionization efficacy of propofol resulting in weak signal intensities. A possible approach to improve the ionization and fragmentation efficacy of this narcotic agent is the conversion of propofol into its DMIS derivative by a derivatization reaction using 1,2-dimethylimidazole-4-sulfonyl chloride (DMISC). Aim of the study is to examine this derivatization reaction in terms of selectivity, linearity, accuracy and precision, analytical limits, processed sample stability and its applicability for biological samples. **Methods:** 200 µl serum was fortified with 20 µl of thymol (1 µg/ml) as an internal standard. Protein precipitation was achieved by addition of 1 ml acetonitrile. 100 µl of 0.1 M sodium bicarbonate buffer (pH 10.5) and 100 µl DMISC (25 mg/ml) were added to 100 µl of the supernatant. Vials were vortexed and allowed to react for 10 min at 60 °C. Subsequently, the reaction mixtures were cooled down to room temperature and extracted twice with 1 ml of n-hexane. The combined organic extracts were evaporated to dryness and redissolved in 100 µl of mobile phase. **Results:** By applying this new derivatization strategy, improvement of the ionization efficiancy of propofol could be achieved and a sufficient number of specific diagnostic fragments of the DMIS derivative of propofol could be detected. Linearity was demonstrated from 5 to 1000 ng/ml with the use of a $1/x^2$ weighting. Accuracy and precision of the method were within in the required ranges. Stability of the processed samples was verified for a time period of up to 25 h. The LoD and LLoQ of the method were determined to be 0.95 ng/ml and 5 ng/ml, respectively. Applicability of the method was confirmed by analysis of a human serum sample collected after propofol-induced sedation. **Discussion:** A new reliable method for the detection of propofol using LC-MS/MS could be developed by using DMISC as derivatization agent. Investigation of recommended validation parameters proved selectivity, linearity, accuracy, precision, processed sample stability and appropriate quantification and detection limits of the developed method. Applicability of the method for biological samples could be confirmed by analysis of a human serum sample collected after propofol-induced sedation. **Conclusion:** A sensitive, robust, and selective LC-MS/MS method for the detection and quantification of propofol in serum was developed and validated using DMISC as derivatization agent.

1. Introduction

Propofol (2,6-diisopropylphenol) is a intravenous anaesthetic that is widely used for induction and maintenance of anesthesia [1], as well as for endoscopic and paediatric sedation [2,3]. Further to its clinical use, propofol is being increasingly misused, particularly by healthcare professionals (4,5]. However, due to well-known side effects of propofol, such as pulmonary

oedema as a consequence of apnoea in the absence of ventilatory assistance or rather rare side effects as e.g. pancreatitis, propofol abuse is accompanied by a high mortality rate [6-10].

Analysis of propofol in biological samples by LC-MS/MS is a great challenge due to weak fragmentation and poor ionization efficacy of propofol resulting in weak signal intensities. In this study, a new approach for propofol derivatization using 1,2-dimethylimidazole-4-sulfonyl chloride (DMISC) with subsequent LC-MS/MS detection is presented. Reliablity of the method is examined in terms of selectivity, linearity, accuracy, precision, analytical limits, processed sample stability and regarding its applicability for biological samples.

2. Material and Methods

2.1. Sample preparation

For sample preparation, 200 µl serum was fortified with 20 µl thymol (1 µg/ml in methanol) as internal standard. Protein precipitation was achieved by addition of 1 ml acetonitrile followed by vortexing and centrifugation (8 min, 1,625 *g*). 100 µl of 0.1 M sodium bicarbonate buffer (pH 10.5) and 100 µl DMISC (25 mg/ml in acetone) were added to 100 µl of the supernatant. Vials were vortexed and allowed to react for 10 min at 60°C. Subsequently, the reaction mixtures were cooled down to room temperature and extracted twice with 1 ml of *n*-hexane. The combined organic extracts were evaporated to dryness on a rotary evaporator and redissolved in 100 µl of mobile phase (75:25, mobile phase A / mobile phase B, v/v).

2.2. LC-ESI-MS/MS analysis

Analyses for propofol-DMIS and thymol-DMIS were carried out by LC-electrospray ionization (ESI)-MS/MS in the multiple reaction monitoring (MRM) mode using two specific ion transitions for each analyte (337.3 → 96.3 and 337.3 → 159.2 for propofol-DMIS and 309.3 → 96.3 and 309.3 → 159.2 for thymol-DMIS). The LC system was equipped with a Phenomenex Luna C8 analytical column (3 mm x 150 mm, 5 µm particle size). The mobile phase consisted of (A) 0.1% (v/v) formic acid with 5 mM ammonium formate and (B) methanol containing 0.01% (v/v) formic acid and 5 mM ammonium formate. A gradient program starting at a composition of 25% B, ramped to 90% B from 1 to 4 min with an isocratic post-run period (4-8 min) with a flow rate of 500 µl/min was applied. Additionally, a re-equilibration step (8-13 min) was implemented to ensure constant retention times of the analytes. The injection volume was 10 µl.

2.3. Method validation

Method validation was carried out in accordance to international guidelines for the analysis of rare analytes under consideration of the following parameters: selectivity, linearity, accuracy, precision, analytical limits and processed sample stability [11,12].

3. Results

3.1. MS/MS analysis

The full-scan mass spectrum of the DMIS derivative of propofol showed an intense $[M+H]^+$ ion at *m/z* 337. The most abundant fragment ions could be observed at *m/z* 96 and *m/z* 159 (Fig. 1). Further product ions could be detected at *m/z* 177 and *m/z* 295.

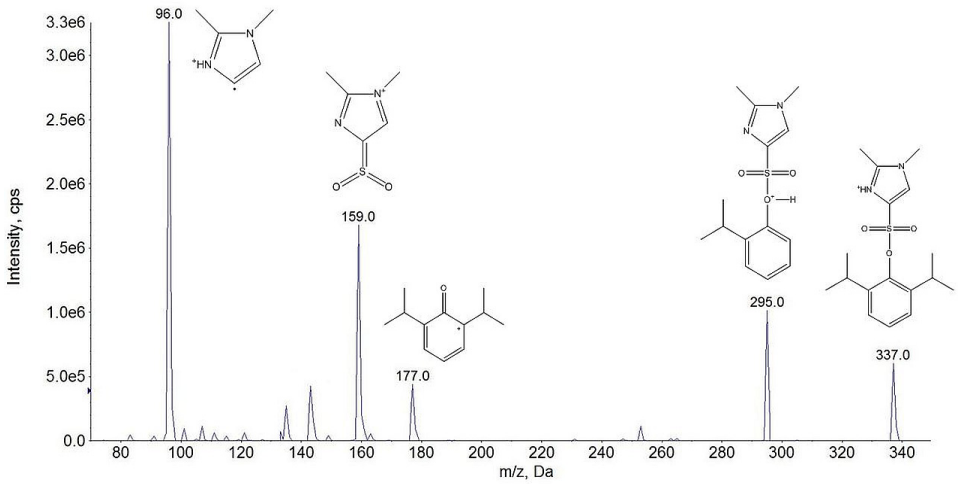

Fig. 1. MS/MS spectrum of propofol-DMIS and proposed product ions' structures.

3.2. Method validation

Chromatographic selectivity of the method was demonstrated by the absence of endogenous interfering peaks at the retention times of propofol-DMIS and thymol-DMIS. The eight-point calibration curves of propofol-DMIS were linear over the concentration range of 5-1000 ng/ml in human serum by using a $1/x^2$ weighting. These curves were used to determine the levels of propofol-DMIS in the serum samples that had been spiked at levels of 20 ng/ml and 700 ng/ml in context of accuracy and precision investigations. Accuracy of the method was evaluated by the bias as the percent deviation of the mean calculated value from the nominal value and was within a range of ±15%. Precision was evaluated by the relative standard deviation and calculated precision data was within 15% RSD. The LoD and LLoQ of the method was determined to be 0.95 ng/ml and 5 ng/ml, respectively. Maximum declines of the peak areas of processed samples at low and high concentrations relative to the calibration range over the tested time period were within the required range, confirming the stability of the processed samples for a time interval of at least 25 hours.

3.3. Applicability for biological samples

A 17-year-old male suffered critical head injuries as a result of a physical altercation. In the framework of intensive medical treatment, he received propofol as a sedative. A serum sample was taken for toxicological analysis in connection with the diagnostic procedure of brain death. Analysis of the serum sample using the described method revealed a propofol concentration of 54.1 ng/ml (Fig. 2).

4. Discussion

The full-scan mass spectrum of propofol solution following derivatization with DMISC revealed an intense peak at *m/z* 337 that can be assigned to the DMIS derivative of propofol. Analysis of the product ion spectrum showed two intense ions at *m/z* 96 and *m/z* 159 presumably representing the dimethylimidazole moieties of this DMIS derivative. Ions of *m/z* 177 and *m/z* 295 observed in the product ion spectrum are likely to be attributed to the deprotonated propofol after elimination of the dimethylimidazolesulfonyl group and the DMIS derivative of 2-isopropyl phenol caused by elimination of the isopropyl group, respectively. Evaluation of the validation data approved the reliability of the method for detection and quantification of propofol in serum after derivatization with DMISC.

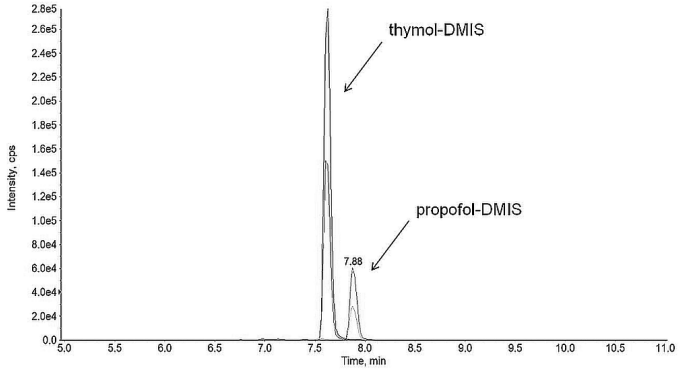

Fig. 2. Chromatogram of a human serum sample collected after propofol-induced sedation. MRM transitions *m/z* 337 → 96 and *m/z* 337 → 159 were used for propofol-DMIS and *m/z* 309 → 96 and *m/z* 309 → 159 for the internal standard thymol-DMIS.

Besides evaluation of selectivity, linearity, accuracy, precision and processed sample stability, appropriate quantification and detection limits (LLoQ = 5 ng/ml; LoD = 0.95 ng/ml) for toxicological analysis of propofol could be achieved, enabling to detect even small quantities of propofol in serum samples. Moreover, the method was also successfully applied to the analysis of a human serum sample collected after administration of propofol in the framework of intensive medical treatment approving the applicability of the method for real human serum samples.

5. Conclusion

A sensitive, robust and selective LC-MS/MS method for the detection and quantification of propofol in serum was developed and validated. This method met regulatory requirements for selectivity, linearity, accuracy and precision, analytical limits and processed sample stability. Applicability of the method to biological samples could be confirmed by analysis of a human serum sample collected after propofol-induced sedation.

6. References

1. Kotani Y, Shimazawa M, Yoshimura S, Iwama T, Hara H. The experimental and clinical pharmacology of propofol, an anesthetic agent with neuroprotective properties. CNS Neurosci Ther. 2008;14(2):95–106.
2. Heuss LT, Schnieper P, Drewe J, Pflimlin E, Beglinger C. Safety of propofol for conscious sedation during endoscopic procedures in high-risk patients - a prospective, controlled study. Am J Gastroenterol. The American College of Gastroenterology; 2003;98(8):1751–7.
3. Lamond DW. Review article: Safety profile of propofol for paediatric procedural sedation in the emergency department. Emerg Med Australas. 2010;22(4):265–86.
4. Strehler M, Preuss J, Wollersen H, Madea B. Lethal mixed intoxication with propofol in a medical layman. Arch für Kriminologie. 2005;217(5–6):153–60.
5. Bell DM, McDonough JP, Ellison JS, Fitzhugh EC. Controlled drug misuse by Certified Registered Nurse Anesthetists. J Am Assoc Nurse Anesth. 1999;67(2):133–40.
6. Boudoin Z. General anaesthetics and anaesthetic gases. In: Dukes MNG, Aronson JK, editors. Meyler's side effects of drugs, 14th edn. Amsterdam: Elsevier; 2000. p. 300.
7. Iwersen-Bergmann S, Rösner P, Kühnau HC, Junge M, Schmoldt A. Death after excessive propofol abuse. Int J Legal Med. 2001;114(4–5):248–51.
8. Drummer OH. A fatality due to propofol poisoning. J Forensic Sci. 1992 Jul;37(4):1186–9.
9. Cirimele V, Kintz P, Doray S, Ludes B. Determination of chronic abuse of the anaesthetic agents midazolam and propofol as demonstrated by hair analysis. Int J Legal Med. 2002;116(1):54–7.
10. Roussin A, Mirepoix M, Lassabe G, Dumestre-Toulet V, Gardette V, Montastruc J-L, et al. Death related to a recreational abuse of propofol at therapeutic dose range. Br J Anaesth. 2006 Aug;97(2):268.
11. Peters FT, Drummer OH, Musshoff F. Validation of new methods. Forensic Sci Int. 2007;165(2–3):216–24.
12. Peters FT, Hartung M, Herbold M, Schmitt G, Daldrup T, Mußhoff F. Anhang B - Anforderungen an die Validierung von Analysenmethoden. Toxichem Krimtech. 2009;79:185–208.