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Determination of atracurium, cisatracurium and mivacurium with their impurities in pharmaceutical preparations by liquid chromatography with charged aerosol detection

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ABSTRACT

The Corona CAD (charged aerosol detection) is a new type of detector introduced for LC applications that has recently become widely applied in pharmaceutical analysis. The Corona CAD measures a physical property of analyte and responds to almost all non-volatile species, independently of their nature and spectral or physicochemical properties. The LC method with charged aerosol detection was developed for the determination of three isomers of atracurium, cisatracurium and also three isomers of mivacurium with their impurities. The limit of quantitation for laudanosine was 1 $\mu g\,ml^{-1}$. The elaborate method for the analysis of those active substances and laudanosine proved to be fast, precise, accurate and sensitive. All other impurities were identified using time-of-flight mass spectrometry with electrospray ionization.

1. Introduction

Atracurium besilate and its 1R-*cis*, 1R′-*cis* isomer, cisatracurium besilate, are non-depolarizing neuromuscular blocking drugs of intermediate duration of action, widely used clinically [1]. Due to four chiral atoms and symmetric structure atracurium possesses 10 stereoisomers. Cisatracurium is a more potent (3–5-fold) isomer of atracurium and has lower histamine releasing potential in clinical doses. Both drugs undergo Hofmann elimination [2,3], a nonenzymatic process dependent on pH and temperature, yielding laudanosine and quaternary monoacrylate. In acidic solutions (pH < 3) atracurium degrades also by ester hydrolysis, where the monoquaternary alcohol and monoquaternary acid are the primary products (Fig. 1). Unlike atracurium, cisatracurium does not appear to be degraded directly by ester hydrolysis.

Mivacurium is a mixture of three isomers (*trans–trans*, *cis–trans*, and *cis–cis*) [4]. It has a short to intermediate duration of action and is hydrolyzed to monoquaternary alcohol and acid (Fig. 2). Opposite to atracurium, its *trans–trans* isomer is more potent than the other ones.

For the determination of atracurium, cisatracurium, mivacurium and other muscle relaxants alone without their impurities or metabolites various methods have been reported: fluorimetric [5], electrochemical [6] and liquid chromatographic (LC) with the following types of detection: UV [7,8], fluorimetric [9–11], NMR [12] and recently electrospray ionization (ESI)-MS/MS [13–15]. Crimele et al. [13] developed a procedure for eight quaternary nitrogen muscle relaxants, including atracurium and mivacurium in blood using LC–MS. A general screening method of 20 quaternary ammonium drugs in equine urine (including only mivacurium of our interest) was worked out after SPE (solid-phase extraction) by LC–MS/MS [14]. A similar LC–MS/MS procedure for determination of 8 quaternary ammonium drugs (e.g. atracurium and mivacurium) and herbicides in human whole blood after weak cation exchange SPE was developed by Arrifin and Anderson [15]. Although the above methods can be used in simultaneous determination of quaternary ammonium drugs, yet it is necessary to detect and determine their degradants and metabolites in the same procedure. Of the published methods mainly LC with fluorimetric detection was used for determination of mivacurium in combination with their metabolites in human plasma after SPE [16] and without extraction [17], for determination of atracurium with laudanosine as the main degradant [18], and cisatracurium with laudanosine [19] and monoquaternary alcohol [20]. Atracurium

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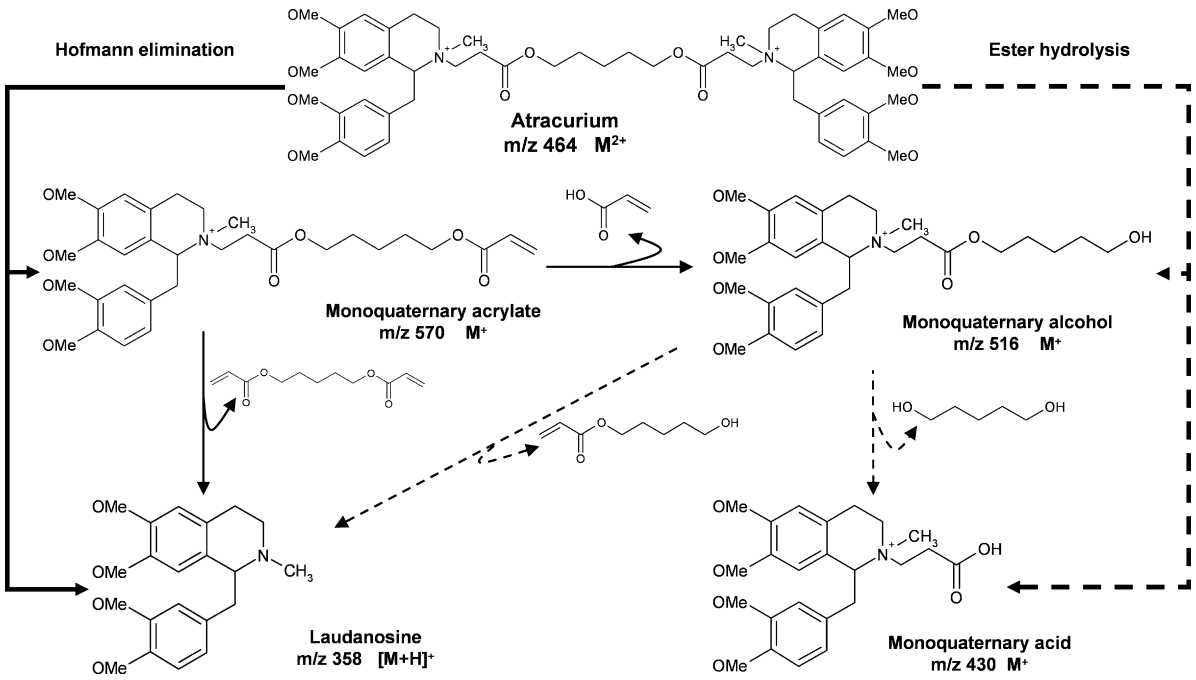

Fig. 1. Degradation pathways of atracurium.

and cisatracurium with their metabolites were also determined using LC–MS [21–24]. However, some of the published methods either did not report assay validation [14,19,21,23] or else reported incomplete assay validations – only for the main compound [24]. Most of them were unable to perform simultaneous determination of isomers of atracurium and its metabolites. The published stereoselective assays for the determination of isomers of atracurium without its metabolites used LC–NMR [12], but the procedure is time-consuming and expensive. When diastereoisomers differ in pharmacological properties, it is very important to have a stereoselective method for analysis. European Pharmacopoeia 6.5 (Ph. Eur.) recommends LC–UV for determination of the isomers of atracurium and its impurities, however, the time of analysis is very long – 50 min [25].

The purpose of this work was to develop a quick and sensitive LC method with charged aerosol detection (CAD) for the determination of three isomers of atracurium, cisatracurium, three isomers of mivacurium, and their degradants, which could be applied to their analysis in substances and pharmaceutical preparations.

The Corona CAD is a new type of detector [26,27] introduced for LC applications and has recently become widely used in pharmaceutical analysis [28–32]. The Corona CAD measures a physical property of analyte and responds to almost all non-volatile species, independently of their nature and spectral or physico-

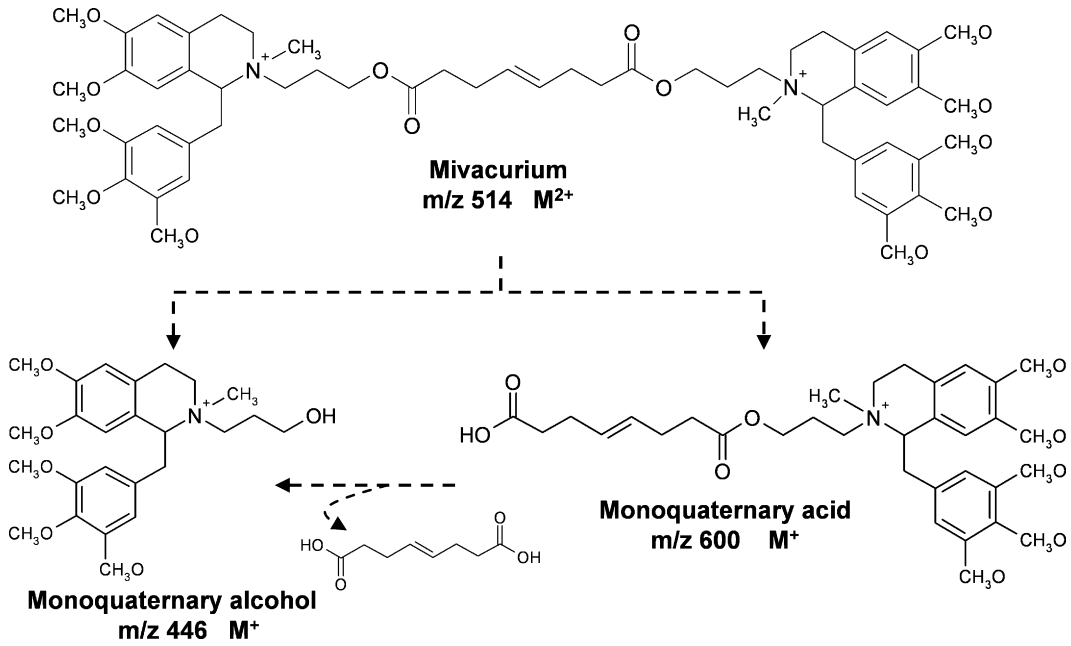

Fig. 2. Degradation pathways of mivacurium.

chemical properties. Other examples of universal detectors used in liquid chromatography to detect compounds with weak or no chromophores include: refractive index (RI) and evaporative light scattering (ELSD). RI detection, however, has significant limitations in sensitivity and reproducibility and it cannot be used in gradient elution analysis. The ELSD is claimed to be less sensitive and not very precise in comparison with the CAD. In the CAD, the effluent from the LC column is first nebulized with nitrogen and the droplets are dried to remove the mobile phase, producing analyte particles. These steps are similar to those of ELSD. The next stage is the ionization of the aerosol by impacting with the positively charged nitrogen obtained by Corona discharge source. The Corona CAD is different from atmospheric pressure chemical ionization (APCI), because the particle is charged, individual molecules are not ionized (charging of particles is not a function of relative proton affinity). This charge from Corona electrode transfers to the opposing stream of analyte particles and is then detected by a sensitive electrometer. The signal is in direct proportion to the quantity of analyte present.

However, one should keep in mind that the Corona detector does not respond to volatile compounds, and the response to compounds of intermediate volatility can be inconsistent. The Corona detector should probably be used in addition to other detectors rather than instead of them.

2. Experimental

2.1. Equipment and conditions

A Corona CAD instrument (ESA, Chelmsford, MA, USA) was equipped with an LC Ultimate 3000 system (Dionex, Germering, Germany) consisting of: a pump, a degasser, an autosampler, a column heater and a pulse damper. Data processing was carried out with Chromeleon 6.8 software (Dionex). Nitrogen gas from nitrogen generator N2-MISTRAL-4 (Schmidlin-DBS, Switzerland), regulated at 35 psi, was introduced to the detector and the resultant gas flow rate was regulated automatically and monitored by the CAD device. Response range was set to 50 pA full scale. Chromatographic analysis was carried out at 25 °C. The analysis was performed on a C_{18} analytical column (Hypersil GOLD, 150 mm × 4.0 mm; 3 μm particle size; Thermo Fisher Scientific, Waltham, MA, USA) with a guard column (Hypersil GOLD, 10 mm × 4.0 mm; 3 μm particle size; Thermo Fisher Scientific). The linear gradient elution was performed using 0.1% formic acid in water (solvent A) and 0.1% formic acid in methanol (solvent B). The gradient increased linearly from 30% to 50% B in 8 min (from the second to the tenth minute) with a hold time of 5 min at the end. The flow rate was 0.5 ml min^{-1} and the injection volume was 10 μl.

A mass spectrometer MicrOTOF-QII from Bruker Daltonics (Billerica, MA, USA) was used to obtain the electrospray ionization time-of-flight mass spectra (ESI-TOF-MS), when peak identifications were required. The following settings were used: electrospray ionization (ESI) in the positive ion mode, dry gas flow rate was set at 9.0 l min^{-1} and the dry heater at 190 °C. The capillary voltage was set at 4500 V and end plate offset at −500 V. MS data were recorded in the full scan mode (from 50 to 800 m/z).

2.2. Material studied

Reference standards: atracurium besilate (Abbott Laboratories), which is a mixture of the *cis–cis*, *cis–trans* and *trans–trans* isomers of 2,2′-[pentane-1,5-diylbis[oxy(3-oxopropane-1,3-diyl)]]bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium] dibenzenesulphonate; cisatracurium besilate (GlaxoSmithKline) – 1R-*cis*, 1R′-*cis* isomer of atracurium; laudanosine (LGC Standards) – 1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline.

Pharmaceutical preparations: Nimbex, a solution for injections and infusions containing 2 mg ml^{-1} of cisatracurium (GlaxoSmithKline); Tracrium, a solution for injections and infusions containing 10 mg ml^{-1} of atracurium besilate (GlaxoSmithKline); Mivacron, a solution for injections containing 2 mg ml^{-1} of mivacurium chloride (GlaxoSmithKline).

Methanol from Labscan (Dublin, Ireland), formic acid from Park Scientific Limited (Northampton, UK), trifluoroacetic acid from Biosolve (Valkenswaard, The Netherlands) – all of them of purity suitable for LC, doubly distilled water additionally purified in the Nanopure Diamond UV Deionization System from Barnstead (Dubuque, IA, USA) were used throughout.

2.3. Standard solutions

Stock standard solutions of atracurium, cisatracurium and laudanosine were prepared weekly. The solvent containing 0.1% formic acid in water was used throughout to dissolve the examined substances and to dilute standard solutions. Approximately 10 mg of each active substance (atracurium and cisatracurium) and 5 mg of the impurity – laudanosine were weighed accurately into a 10-ml volumetric flask and dissolved with the solvent mentioned above. These solutions were further successively diluted with the solvent to obtain the required concentrations. All solutions were stored in a cool, dark place when not in use.

3. Results and discussion

3.1. Selection of chromatographic conditions

To obtain optimal chromatographic separation, different mobile phases in isocratic and gradient elution were evaluated. For analytical purposes, the gradient elution was much better for separation of atracurium or mivacurium isomers and impurities from each other. Mobile phases with 0.1% trifluoroacetic acid (pH 2) and 0.1% formic acid (pH 3) were evaluated. However, when trifluoroacetic acid was used, the retention times were longer, the noise was higher and the signal-to-noise ratio (S/N) was lower (S/N was equal to 4.8 and 33.2 for trifluoroacetic and formic acid, respectively).

The best response was obtained with a C_{18} analytical column (150 mm × 4.0 mm, 3 μm particle size; Thermo) and the mobile phase containing 0.1% formic acid in water (solvent A) and 0.1% formic acid in methanol (solvent B) in gradient elution. The following gradient was used: 0–2 min 30% B, 2–10 min 30–50% B, 10–15 min 50% B, before returning to the initial conditions at 17 min. The column was equilibrated (in 30% B) for 3 min at the end of each run.

The effect of different flow rates (from 0.4 to 0.6 ml min^{-1}) and column temperature (from 20 to 30 °C) on the peak area and resolution was studied. The flow rate was set at 0.5 ml min^{-1} and the temperature of 25 °C was used throughout. These conditions were optimal for the separation of atracurium and mivacurium isomers and their degradants from each other with the resolution values ranging from 2 to 18. All retention times, resolution values, tailing factors and numbers of plates are presented in Table 1.

The above chromatographic conditions were finally chosen for further investigations.

3.2. Identification of analytes

Standards of mivacurium and degradants of atracurium, cisatracurium and mivacurium like monoquaternary acids, monoquaternary alcohols (of ester hydrolysis) or monoquaternary acrylates (of Hofmann elimination) were not available for us to

Table 1
Observed *m/z* values and chromatographic (LC-CAD) parameters for atracurium and its impurities.

	m/z	Retention time (min)	Resolution	Tailing factor	Plates
Monoquaternary acid *trans*	430 M^+	4.56	2.28	1.38	11,835
Monoquaternary acid *cis*	430 M^+	4.79	8.70	0.97	10,427
Laudanosine	358 $[M+H]^+$	5.87	7.57	1.20	31,762
Monoquaternary alcohol *trans*	516 M^+	7.19	5.26	1.14	49,945
Monoquaternary alcohol *cis*	516 M^+	8.31	2.19	1.07	67,572
Atracurium *trans–trans*	464 M^{2+}	9.18	2.16	1.69	34,634
Atracurium *cis–trans*	464 M^{2+}	9.83	2.02	1.80	56,687
Atracurium *cis–cis*	464 M^{2+}	10.36	18.11	1.55	68,028
Monoquaternary acrylate *trans*	570 M^+	13.94	3.21	1.05	119,170
Monoquaternary acrylate *cis*	570 M^+	14.49	–	1.00	101,402

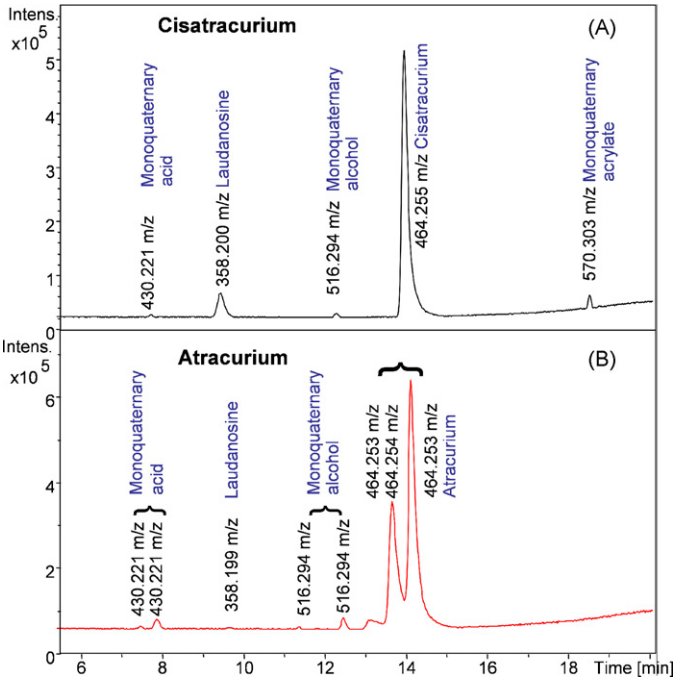

Fig. 3. Mass chromatograms (ESI in positive mode) recorded for peak identification from the solutions containing 10 μg ml^{-1} of cisatracurium (A) and 10 μg ml^{-1} of atracurium (B), injection volume 1 μl.

prepare standard stock solutions for identification and quantitation purposes. However, because in LC-CAD and LC–MS the same volatile mobile phases can be used, the elaborate LC-CAD method for chromatographic separation was transferred into LC–ESI-TOF-MS. By this analysis unknown peaks were identified as products corresponding to degradation pathways of cisatracurium (Fig. 3A), atracurium (Fig. 3B) and mivacurium (Fig. 4). It was transferred to LC-CAD and similar chromatograms were obtained (Fig. 5).

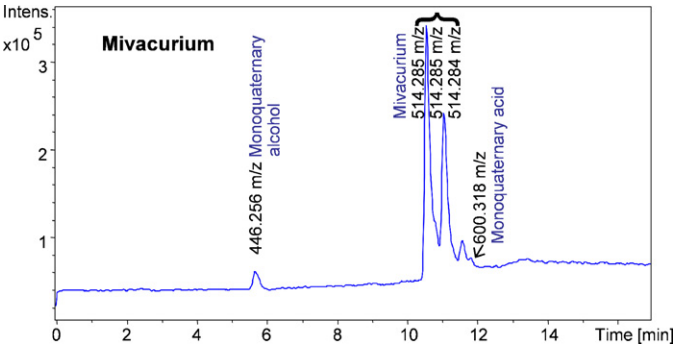

Fig. 4. Mass chromatogram (ESI in positive mode) recorded for peak identification from the solutions containing 10 μg ml^{-1} of Mivacron, injection volume 1 μl.

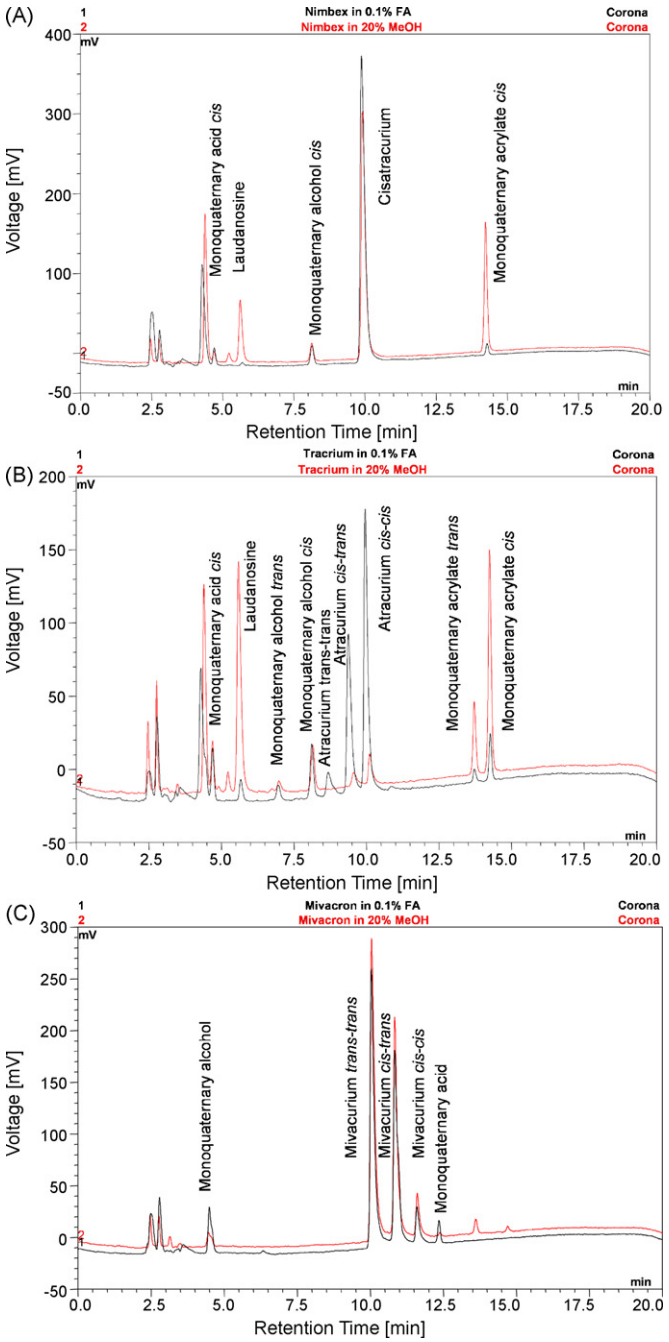

Fig. 5. LC-CAD chromatograms recorded from pharmaceutical preparations containing ca. 100 μg ml^{-1} of atracurium from Tracrium (A), cisatracurium from Nimbex (B) and mivacurium from Mivacron (C) in 0.1% formic acid (black) and in 20% methanol (red); injection volume 10 μl. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 2
Linear and exponential fit equations for calibration curves for atracurium, cisatracurium and laudanosine determined by LC-CAD.

	Range (μg ml^{-1})	Linear		Power		Log–log	
		$y = ax + b$	r^2	$y = ax^b$	r^2	$\log y = b \log x + \log a$	r^2
Atracurium (3 isomers)	1–150						
cis–cis		$0.3232x - 0.6894$	0.9986	$0.3740x^{0.98}$	0.9996	$0.9803x - 0.4366$	0.9996
cis–trans		$0.2158x - 0.0368$	0.9997	$0.1761x^{1.05}$	0.9991	$1.050x - 0.7542$	0.9991
trans–trans		$0.0381x - 0.7320$	0.9986	$0.0068x^{1.32}$	0.9961	$1.324x - 2.170$	0.9961
Cisatracurium	1–150	$0.5172x + 2.6031$	0.9926	$1.032x^{0.87}$	0.9995	$0.8725x + 0.0139$	0.9995
Laudanosine	1–10	$0.7362x - 0.0351$	0.9992	$0.6749x^{1.04}$	0.9986	$1.043x - 0.1708$	0.9986

3.3. Validation of the methods

The quantitative aspects of the proposed methods were examined according to ICH guidelines [33]. The statistical evaluation for all analyzed substances was calculated using Chromeleon Validation ICH software. The data concerning method validation are summarized in Tables 2–5. Peak areas were evaluated in the whole validation.

Except laudanosine, the other degradants of atracurium, cisatracurium and mivacurium were not available for us to prepare standard stock solutions for calibration purposes. They could not, therefore, be quantitatively determined. Because of universal response of Corona CAD to almost all non-volatile species, independently of their nature and spectral or physicochemical properties, even unidentified impurities or the analytes for which no pure standards are available can be quantified in isocratic elution.

3.3.1. Linearity

The linearity was estimated by analyzing atracurium, cisatracurium and laudanosine standards. Several (at least 5) concentrations of the analyzed substances ranging from 5 to 150 μg ml^{-1} (for active substances) and from 1 to 15 μg ml^{-1} (for laudanosine) were used to obtain calibration curves. The 1 μg ml^{-1} concentration standard of atracurium and cisatracurium were excluded, because peak areas were lower than LOQ. A linear response was not expected, since aerosol charging does not depend directly on the aerosol mass [27]. For cisatracurium the response of the Corona detector was not linear (Table 2), but good linearity was obtained when the curve was plotted as a power function $y = ax^b$, where y is the response of the Corona CAD (peak area), x is the concentration of the sample, and a and b are coefficients that depend on droplet size, nature of solute, gas and liquid flow rates, molar volatility, etc. [34]. A double logarithmic coordinate system was used to obtain a linear calibration curve $\log y = \log a + b \log x$, with a good linear fit (Table 2).

Although it is known that Corona CAD response is nonlinear within the range of four orders of magnitude, we found that the signal is nearly linear in the examined ranges of other analyzed compounds [34].

3.3.2. Detection and quantitation limits

3.3.2.1. Based on signal-to-noise ratio. Determination of the signal-to-noise ratio (S/N) was performed by comparing the measured signals from samples of known low concentrations of the analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. The detection limit (LOD) and quantitation limit (LOQ) were defined as signal-to-noise ratio of 3:1 and 10:1, respectively. The lowest LOD and LOQ were obtained for the impurity – laudanosine, and were equal to 0.5 μg ml^{-1} (LOD) and 1.6 μg ml^{-1} (LOQ). For the other analyzed substances, their limits of detection and quantitation are presented in Table 3.

3.3.2.2. Based on the standard deviation of the response and the slope. The detection and quantitation limits were also determined as: $3.3\sigma/S$ and $10\sigma/S$, respectively, where σ is the standard deviation (SD) of the response and S is the slope of the calibration curve. The slope S was estimated from the calibration curve of the analyte. The estimation of σ was carried out in two ways: based on SD of the blank and based on the calibration curve. For the first of them, the measurement of the magnitude of analytical background response was performed by analyzing numerous blank samples and calculating the standard deviation of these responses. For the other one, a specific calibration curve was studied using samples containing an analyte in the range of LOQ.

Several approaches for determination of the detection and quantitation limits are possible [33], but the results obtained in our study vary (Table 3). The approach based on SD of the blank proved to be the most sensitive while S/N approach was the least sensitive. The smallest LOD/LOQ values calculated for the approach based on SD of the blank could not be in most cases verified experimentally, so visual evaluation was done for justification and the data were close to those obtained from the S/N approach. 1 mV noise was detected in the study.

These results are in accordance with the literature [33], where the approach based on S/N was suggested to analytical procedures which exhibit constant baseline noise, i.e. in chromatographic methods. In the case of spectrophotometric methods LOD and LOQ are determined using the approaches based on SD.

Table 3
Limits of detection (LOD) and limits of quantitation (LOQ) for atracurium, cisatracurium and laudanosine determined by LC-CAD.

	Range (μg ml^{-1})	S/N		SD of the response and the slope				S/NSD of the blankcalibration	
		LOD	LOQ	SD of the blank		Calibration curve		LOD	LOQ
				LOD	LOQ	LOD	LOQ		
Atracurium (3 isomers)									
cis–cis	1–150	1.496	4.987	0.647	1.961	0.780	2.363	1.0	3.0
cis–trans	5–150	3.912	13.04	1.460	4.425	1.209	3.662	2.0	6.0
trans–trans	50–150	19.50	65.01	10.81	32.75	5.139	15.57	15.0	50.0
Cisatracurium	1–15	0.660	2.198	0.387	1.172	0.779	2.360	0.8	2.5
Laudanosine	1–10	0.499	1.664	0.267	0.810	0.305	0.923	0.3	1.0

Table 4
Precision and accuracy for atracurium, cisatracurium and laudanosine determined by LC-CAD.

Substance	Concentration added ($\mu g\,ml^{-1}$)	Concentration found ± confidence level ($\mu g\,ml^{-1}$)	SD	RSD intra-day (%)	RSD inter-day (%)	Recovery (%)
Atracurium (3 isomers)						
cis–cis isomer	78.24	79.56 ± 0.20	0.444	0.558	0.808	101.63
	97.80	97.67 ± 0.58	0.233	0.239	0.539	99.86
	117.36	116.36 ± 0.44	0.179	0.154	0.315	99.06
cis–trans isomer	78.24	78.23 ± 1.87	0.751	0.960	1.034	100.80
	97.80	96.80 ± 1.62	0.652	0.673	0.526	100.00
	117.36	116.13 ± 1.21	0.487	0.419	0.355	99.84
trans–trans isomer	78.24	76.32 ± 3.04	1.224	1.604	0.340	96.50
	97.80	97.24 ± 3.41	1.373	1.412	1.756	99.23
	117.36	118.59 ± 4.21	1.694	1.429	2.092	100.25
Cisatracurium	77.60	78.12 ± 1.09	0.848	0.560	0.624	99.71
	96.99	95.84 ± 0.24	0.452	0.100	0.347	100.45
	116.39	116.91 ± 1.70	0.446	0.584	0.373	99.90
Laudanosine	2.99	3.07 ± 0.08	0.034	1.106	2.737	100.25
	4.98	5.18 ± 0.09	0.037	0.715	1.267	100.26
	7.97	8.06 ± 0.14	0.055	0.681	1.279	98.87

3.3.3. Precision and accuracy

Repeatability was assessed using three concentrations covering the specified range for the procedure. The precisions were calculated from three consecutive injections for each concentration and the observed RSD ranged from 0.10 to 1.60% (Table 4). Intermediate precision was calculated from 2 days. The accuracy of the recovery for atracurium, cisatracurium and laudanosine was evaluated at three concentrations. The mean recoveries for all samples from each run were in the range of 96.5–101.6% (Table 4).

3.3.4. Range

The calibration curves for the response of atracurium and cisatracurium in the range from 80 to 120 $\mu g\,ml^{-1}$, corresponding to 80–120% of the assay concentration level of 100 $\mu g\,ml^{-1}$ were performed. Responses obtained in the examined range can be expressed by a linear equation $y = ax + b$ with good r^2 correlation coefficient values ($y = 0.5602x + 3.4038$, $r^2 = 0.9988$; $y = 0.5062x + 6.6285$, $r^2 = 0.9970$ for atracurium and cisatracurium, respectively). For laudanosine also linear response in the examined range 1–10 $\mu g\,ml^{-1}$ was obtained ($y = 0.7362x - 0.0351$, $r^2 = 0.9992$).

It was confirmed that when the level is very low or when the range is small, the calibration curve is close to a linear curve [34].

3.4. Determination of active substances and impurities in pharmaceutical preparations

0.8 ml of Nimbex solutions containing cisatracurium and 2.0 ml of Tracrium solutions containing atracurium were transferred into 20-ml volumetric flasks and diluted with 0.1% formic acid and were further successively diluted with 0.1% formic acid to the concentration of ca. 100 $\mu g\,ml^{-1}$ of the active substance. For laudanosine determination 5 ml of Nimbex and 1.0 ml of Tracrium were transferred into 10-ml volumetric flasks and diluted with 0.1% formic acid to the concentration of ca. 1 $mg\,ml^{-1}$ of the active substance.

Thus prepared sample solutions were used for qualitative studies. In both determinations six prepared samples were used. Then 10 μl of solutions was introduced into the column and the chromatograms were recorded for 20 min. The contents were determined by the calibration curve method. All data are summarized in Table 5. The *cis–cis* isomeric group constituted 58.28%, *cis–trans* 36.66%, and *trans–trans* isomer 5.06% of the atracurium mixture.

The other impurities could not be determined due to lack of standards, however, the determination should be possible when using a second pump for mobile-phase compensation.

3.5. Stability

In vitro investigations in various buffers and plasma [2,3] suggest that cisatracurium and atracurium undergo temperature- and pH-dependent Hofmann elimination yielding laudanosine and a quaternary monoacrylate (Fig. 1). Due to ester hydrolysis of atracurium and mivacurium monoquaternary acids and alcohols are formed (Figs. 1 and 2). This was confirmed in our study. The analyzed active substances and pharmaceutical preparations were also dissolved in 20% methanol. The degradation was very fast. As we expected, for atracurium the contents of all possible degradants from Hofmann elimination and ester hydrolysis increased in Tracrium sample, whereas the content of atracurium sharply decreased (Fig. 5A). In the case of cisatracurium (Nimbex)

Table 5
Determination of active substances and impurities in pharmaceutical preparations by LC-CAD.

		Declared amount	Found amount	% of declared amount	SD	RSD (%)
Tracrium	Atracurium (3 isomers)	10.0 $mg\,ml^{-1}$	10.83 ± 0.05 $mg\,ml^{-1}$	108.35	0.044	0.407
	cis–cis isomer	55.0–60.0%	58.28 ± 0.17%		0.166	0.285
	cis–trans isomer	34.5–38.5%	36.66 ± 0.14%		0.134	0.366
	trans–trans isomer	5.0–6.5%	5.06 ± 0.10%		0.097	1.918
	Laudanosine	Max. 1.0%[a]	0.50 ± 0.01%		0.334	0.677
Nimbex	Cisatracurium	2.68 $mg\,ml^{-1}$	2.87 ± 0.03 $mg\,ml^{-1}$	107.14	0.017	0.595
	Laudanosine	Max. 1.0%[a]	0.56 ± 0.05%		0.108	0.969

[a] For substance atracurium besilate, not for pharmaceutical preparation.

only the contents of laudanosine and monoquaternary acrylate slightly increased, while the content of cisatracurium decreased (Fig. 5B). For the Mivacron sample higher contents of monoquaternary acid and alcohol were observed (Fig. 5C). To estimate monoquaternary acid, alcohol and monoacrylate formations, the peak areas (as obtained from the LC chromatograms) were compared.

It is crucial, therefore, to prepare the stock solutions of atracurium, cisatracurium and mivacurium in a slightly acidic solution (pH 3), in our study in 0.1% formic acid, to avoid acidic hydrolysis and Hofmann elimination. Thus prepared solutions were stable for at least 2 weeks when stored at +6 °C (±2 °C). After 2 months of storage the content of atracurium decreased by almost 10%, while the contents of monoquaternary acid and alcohol slightly increased.

4. Conclusion

In this work, the LC-CAD method for the determination of three isomers of atracurium, cisatracurium and three isomers of mivacurium with their impurities was developed to provide a sensitive, quantitative assay of active substances and their impurity – laudanosine in substance and in pharmaceutical preparations. The conditions were optimized so as to obtain the best signal and stability of the measurement with the highest sensitivity. The preparation of samples and their analysis were performed within a relatively short time. These elaborate methods for the analysis of atracurium, cisatracurium with their impurities proved to be fast, precise, accurate and sensitive, and could be applied to routine analysis in substances and in pharmaceutical preparations. Mivacurium and all impurities were identified using time-of-flight mass spectrometry with electrospray ionization.

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