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Determination of dextropropoxyphene and nordextropropoxyphene in urine by liquid chromatography–electrospray ionization mass spectrometry

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Abstract

Dextropropoxyphene and nordextropropoxyphene were extracted from urine samples with mixed mode solid-phase extraction cartridges. After elution and evaporation to dryness, the eluate was dissolved in mobile phase and each sample was injected in a LC–ESI-MS system. Quantification was carried out in the selected ion monitoring mode. This article shows the possibility to analyse drugs of abuse substances in urine with a single quadrupole mass spectrometer if only a thorough work-up procedure and a sufficient chromatographic separation is accomplished. In order to enhance the fragmentation of the analytes, in-source fragmentation was carried out. One fragment and the pseudomolecular ion per analyte together with chromatographic retention times were sufficient to verify that the sought compound was found in the samples. In- and between day variation was lower than 10% and the recovery was well above 90%. The analytes were quantified in the range 100–10 000 ng/ml urine.

Keywords: Dextropropoxyphene; Nordextropropoxyphene

1. Introduction

In our laboratory we analyse urine samples from psychiatric departments, drug treatment programmes etc. to determine whether the samples contain drugs or drugs of abuse. Urine is the usual matrix for analysis of drugs of abuse. Partly because of a greater analysis time-window compared to analysis in plasma/serum, higher drug concentrations and simpler sample collection. However, sometimes the original drug is totally metabolised in urine samples but in many cases, such as the one described here, both the original drug and the active metabolite can be found. One of the drugs in our analysis programme is dextropropoxyphene. Dextropropoxyphene is an analgesic which is used to alleviate mild to moderate pain disorders. The substance is a synthetic weak opioid and is used by drug abusers because of its effect on the central nervous system. However, it is well known that dextropropoxyphene together with alcohol causes lethal overdose conditions, even in moderate overdose. Several ways have been explored for the analysis of dextropropoxyphene and its main active metabolite nordextropropoxyphene [1]. High-performance liquid chromatography (HPLC) combined with liquid–liquid extraction as a work-up procedure has been utilised

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[2–4] and also HPLC with solid-phase extraction (SPE) [5]. In order to simplify the analysis of the metabolite nordextropropoxyphene with gas chromatography (GC), a rearrangement procedure that involves treating the sample with strong base has been performed in several studies [6–9]. Other routes of analysing these substances have been with various immunological screening methods in combination with GC–MS [8–12]. Finally, Fitzgerald et al. [13] used a column switching instrument coupled on-line to an MS–MS system to be able to extract, purify and separate drugs without any prior work-up procedure. Our work will show that identification and quantitation can be done with a single quadrupole LC–MS system when a thorough work-up procedure and a sufficient separation is achieved on the chromatographic column before the MS analysis.

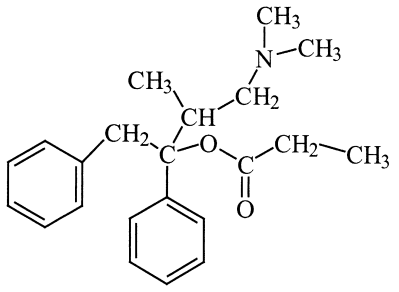
2. Experimental

2.1. Chemicals

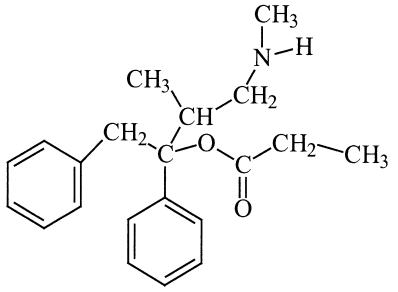
(+)-Dextropropoxyphene in ampoules at a concentration of 1.0 mg/ml in methanol was bought from Cerilliant (LGC Promochem, Borås, Sweden). *d*-Nordextropropoxyphene as the maleate salt, was bought from Sigma (Sigma–Aldrich Sweden, Stockholm, Sweden). Solution at a concentration of 1.0 mg/ml was made by dissolving 13.6 mg of the maleate salt in Milli-Q (Millipore, Sundbyberg, Sweden) water. From these solutions a working standard of 100 μg/ml was made in water and this solution could be kept in the refrigerator for 3 months. The internal standard, (±)-dextropropoxyphene-D_5, was also bought from Cerilliant in ampoules at a concentration of 1.0 mg/ml in methanol (Fig. 1). From this solution, 0.4 ml was added to 9.6 ml of methanol, which gave a concentration of 40 μg/ml in the working solution. The 0.1-*M* phosphate buffer was made from KH_2PO_4 (Merck, VWR International, Stockholm, Sweden) where 13.6 g was added to 900 ml of MilliQ water and the pH adjusted with 1.0 *M* potassium hydroxide to 6.0 before diluting to 1 l. The 1.0-*M* acetic acid was made from 30 ml of glacial acetic acid (Merck) which was diluted to 500 ml with MilliQ water. The extraction solvent, 2% ammonia (Merck) in ethyl acetate (LabScan, Dublin, Ireland), was prepared fresh daily. The urine that was used in these experiments was donated by the staff of the laboratory and was screened for drugs of abuse in our analysis programme by GC–MS and stored in a freezer before it was utilised. All other solvents were of analytical grade.

2.2. Equipment

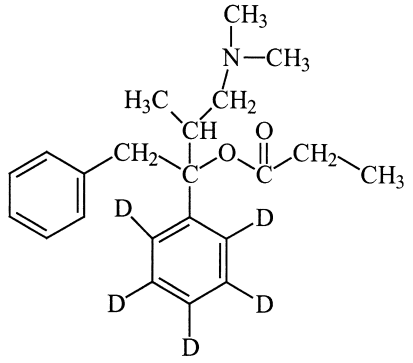
The separation was performed on a ACE 3 AQ column (ACT, Scantec Lab, Partille, Sweden), which basically is a C_{18} column with embedded polar groups which makes it capable to handle mobile phases with high water content. The dimension was 150×2.1 mm, with 3-μm particles and the column was kept at 20 °C during the run in a column oven. The mobile phase for liquid chromatography was 35% acetonitrile/65% 0.1% formic acid in a isocratic run with a column flow of 0.25 ml/min and a total analysis time of 6 min. The solid-phase extraction cartridges were Isolute HCX (IST, Sorbent, Västra Frölunda, Sweden), filled with 130 mg modified silica in a 10-ml reservoir volume. The sorbent in this cartridge is silica based with functional groups consisting of C_8 and SO_3^- which makes it suitable for the extraction of basic analytes with dual retention mechanism. To speed up the extraction process a vacuum manifold, VacMaster (IST), which can process up to 20 samples at a time, was used. A gentle stream of air was used for the evaporation of the organic solvent at the end of the sample preparation, while placing the samples in a heating block (Techne Dri-Block, DB-3D, Techne, Cambridge, UK). The LC–MS system was a Agilent 1100 LC/MSD (G1946D) system equipped with a degasser, quaternary pump, autosampler, column compartment with adjustable temperature and mass spectrometer (Agilent Technologies Sweden, Kista, Sweden). Electrospray-mass spectrometry (ESI-MS) was performed in the positive mode with the following operating parameters: nebulizer pressure 35 p.s.i.g., nebulizer nitrogen gas flow-rate 13 l/min, drying gas temperature 350 °C and a capillary voltage of 4 kV. Optimisation of the fragmentation was done by direct injection of each of the different substances, dissolved in mobile phase, into the mass spectrometer with flow injection analyses (FIA) using the autosampler. In this way the fragmentor or cone voltage



Dextropropoxyphene



Nordextropropoxyphene



Dextropropoxyphene-D_5 (internal standard)

Fig. 1. Structures of dextropropoxyphene, nordextropropoxyphene and the internal standard dextropropoxyphene-D_5.

could be optimised so that at least two ions per substance were measured at the expense of some sensitivity. The following pseudomolecular, $(M+H)^+$, and fragment ions were measured per substance at the specified fragmentor voltage: dextropropoxyphene m/z 340, 266 at 150 V; nordextropropoxyphene m/z 326, 252 at 100 V; dextropropoxyphene-D_5 m/z 345, 271 at 150 V. The relative response between all these pair of ions were determined as 40±10% per substance when the specified fragmentor voltages were used. The criteria for identification were thus chromatographic retention times and relative response between the fragment ion and the pseudomolecular ion. The criteria relative response means that the quotient between the abundance of the fragment ion and the abundance of the pseudomolecular ion should be 40±10% to be able to identify the substance. These criteria's were then used to identify dextropropoxyphene and nordextropropoxyphene in all internal controls and patient samples that were analysed throughout the work.

2.3. Sample preparation

To 0.5 ml urine, 50 μl of an internal standard solution (40 μg/ml methanol) and 2 ml phosphate buffer, pH 6.0, were added and the samples were briefly vortex mixed. The solid-phase extraction cartridges were installed on the vacuum manifold and conditioned with 2 ml of methanol and 2 ml of 0.1 *M* phosphate buffer, pH 6.0, before applying the samples. The urine was allowed to pass the cartridges by gravity before washing the cartridges. Washing was performed with 1 ml of 1 *M* acetic acid

and drying under full vacuum for 5 min followed by 1 ml of acetonitrile and drying for 2 min under full vacuum. The 5-ml glass tubes were placed in the vacuum manifold and trapped components were eluted with 2 ml of 2% ammonia in ethyl acetate. The eluates were then evaporated to dryness by a gentle stream of air while placing the glass tubes in a 40 °C heating block. One ml of mobile phase was used to dissolve the samples in the glass tubes and the tubes were briefly vortex mixed before transferring the solution to 2 ml autosampler vials. The described method is a variant of a standard procedure for extraction of basic analytes, supplied by companies selling extraction equipment [14].

3. Results and discussion

3.1. Precision and recovery

The relative standard deviation (RSD) of the within-day precision ($n=6$) was lower than 5% for both low and high internal control samples. The accuracy was better than ±5% for both analytes. The between-day precision ($n=6$) had slightly higher RSDs with deviations between 6 and 7% for both analytes at the two different levels. The accuracy was however better than ±3% from the target value (Table 1). The recovery for dextropropoxyphene from spiked urine samples was 94% at the two tested levels, 100 and 10 000 ng/ml ($n=6$). For nordextropropoxyphene the recovery was 98 and 106% at the different levels ($n=6$, Table 1). No internal standard was used in this study, instead absolute peak areas were compared between spiked urine samples and samples that theoretically was 100% made in buffer solution.

3.2. Linearity

The linearity of the method was evaluated by analysing six calibration curves in the range 100–10 000 ng/ml and determine the mean value of the slope and intercept with the y-axis. In the straight-line equation, the area ratio of (peak area of the analyte/peak area of the internal standard) can be expressed as y, while x is the concentration ratio of the (analyte in the sample/concentration of the internal standard in the sample). The relative standard deviation (RSD) of the slope was defined as the ratio of standard deviation from six calibration curves/mean value of six slopes multiplied with 100. The equation for dextropropoxyphene was $y = 0.538x - 0.0006$ with a RSD of the slope of 1.1%. For nordextropropoxyphene the equation was $y = 0.545x + 0.008$ with a RSD of the slope of 6.6%. The mean regression coefficient ±standard deviation, for six calibration curves was 0.999±0 for

Table 1
Within- and between-day variation and recovery data ($n=6$)

Analyte	Target (ng/ml)	Measured (ng/ml)	SD (ng/ml)	RSD (%)	Accuracy (±%)	Recovery (%)
Within-day variation						
Dextropropoxyphene	100	103	0.8	0.8	+3.0	
	10 000	9643	217	2.2	−3.6	
Nordextropropoxyphene	100	103	4.8	4.7	+3.0	
	10 000	9718	416	4.3	−2.8	
Between-day variation						
Dextropropoxyphene	100	102	7.0	6.9	+2.0	
	10 000	9734	585	6.0	−2.7	
Nordextropropoxyphene	100	100	6.1	6.1	0	
	10 000	10 072	702	7.0	+0.7	
Recovery						
Dextropropoxyphene	100	94	7.5	7.9	−5.5	94
	10 000	9424	141	1.5	−5.8	94
Nordextropropoxyphene	100	106	8.2	7.8	+6.1	106
	10 000	9839	246	2.5	−1.6	98

dextropropoxyphene and 0.998±0.002 for nordextropropoxyphene.

3.3. Lower limit of quantification

To be able to determine the low limit of quantification (LLOQ), a number of spiked urine samples were processed and analysed with the method. The samples were all spiked in concentrations below the lowest concentration in the standard curve which means concentrations less then 100 ng/ml. Five samples were analysed per concentration level and the criteria for approval was a RSD level less then 20% and accuracy ±20% from target value. Another criteria was that the relative response, between the fragment and pseudomolecular ion, should agree with the method. The data from this analysis can be seen in Table 2 where concentrations down to 20 ng/ml could be measured with good precision and accuracy for dextropropoxyphene. For nordextropropoxyphene this level was 60 ng/ml. Both these values meet our needs as a drug-testing laboratory as we seldom see concentrations in this range in patient materials. The LLOQ for nordextropropoxyphene is somewhat poorer than that for dextropropoxyphene, but this of no real significance since it is well known that the concentration of the metabolite is several times higher in blood and urine after intake of dextropropoxyphene. The LLOQ is often the practical limit of detection (LOD) because peaks below the LLOQ are seldom quantifiable if the criteria of relative response (between the pseudomolecular ion and the fragment ion) are to be followed. This means that the definition of LOD with a signal-to-noise ratio of 3:1 is not applicable here.

Table 2
Determination of lower limit of quantification ($n=5$)

Analyte	Target (ng/ml)	Measured (ng/ml)	SD (ng/ml)	RSD (%)	Accuracy (±%)
Dextropropoxyphene					
	80	78	0.6	0.8	−2.5
	60	60	0.3	0.5	0
	40	42	0.8	1.9	5.0
	20	24	0.8	3.4	20.0
Nordextropropoxyphene					
	80	85	1.6	1.8	6.2
	60	62	0.8	1.3	3.3

3.4. Storage

An important parameter when validating analysis methods is undoubtedly storage of samples in different environments. This gives us knowledge how the analytes behave in the matrix during transportation and how to handle them when they arrive at the laboratory. In our validation programme we store samples at room temperature for 3 days in light and darkness, 7 days in a refrigerator (+4–8 °C) and 3 and 6 months in a freezer (−20 °C). After thawing the samples they are processed and analysed along with standard samples which are freshly spiked for this occasion. In Table 3 the result shows that there is no problem to store urine samples containing dextropropoxyphene and nordextropropoxyphene in room temperature for 3 days, in a refrigerator for 7 days and freezer (−20 °C) for up to 3 months. Between 3 and 6 months in the freezer at −20 °C, the analytes seems to degrade. Especially nordextropropoxyphene shows poor recovery after 6 months storage in a freezer, with 50% recovery at the low control level. This shows that urine samples containing dextropropoxyphene and/or nordextropropoxyphene can be stored in freezers for at least 3 months.

3.5. Selectivity

The robustness of the method was exemplified in two different tests. In the first test two groups of urine samples were spiked with high levels, 10 000 ng/ml, of pharmaceutical compounds as well as low levels, 500 ng/ml, of dextropropoxyphene and nordextropropoxyphene. The substances chosen for this test were those that often can be found in patient samples from psychiatric departments, e.g. antipsychotics, antidepressants and tranquillisers. The spiked samples were then processed and analysed along with standard and control samples and the result is shown in Table 4. Even though the concentration of the disturbing substances are relatively high this does not seem to affect the overall result of the analysis which give precision data lower than 10% and accuracy data better than ±15%. In the second test urine samples from 10 patients were analysed in order to find out if real samples did contain something that could disturb the analysis.

Table 3
Storage of urine samples containing dextropropoxyphene and nordextropropoxyphene ($n=6$)

Condition	Analyte	Target (ng/ml)	Measured (ng/ml)	SD (ng/ml)	RSD (%)	Accuracy (%)
Room temp. light 3 days	Dextropropoxyphene	100	100	0.6	0.6	0.0
		10 000	9641	160	1.7	−3.6
	Nordextropropoxyphene	100	98	11	11.0	−2.0
		10 000	9100	462	5.1	−9.0
Room temp. darkness 3 days	Dextropropoxyphene	100	100	1.3	1.3	0.0
		10 000	9471	182	1.9	−5.3
	Nordextropropoxyphene	100	83	10.4	12.5	−17.0
		10 000	9179	272	3.0	−8.2
Refrigerator +4–8 °C, 1 week	Dextropropoxyphene	100	104	1.2	1.2	4.0
		10 000	10 095	180	1.8	1.0
	Nordextropropoxyphene	100	99	11.4	11.5	−1.0
		10 000	9916	450	4.5	−0.8
Freezer −18 °C 3 months	Dextropropoxyphene	100	90	1.6	1.8	−10.0
		10 000	9216	141	1.5	−7.8
	Nordextropropoxyphene	100	110	23.7	21.5	10.0
		10 000	11 457	787	6.9	11.4
Freezer −18 °C 6 months	Dextropropoxyphene	100	77	1.1	1.4	−23
		10 000	9426	79	0.8	−5.7
	Nordextropropoxyphene	100	51	1.8	3.6	−51
		10 000	7241	580	8.0	−28

The samples chosen were those that the laboratory had received for the analysis of metabolites of cannabis. We felt that those samples were the best candidates in this study in the way that they were not likely to contain any dextropropoxyphene. The result of the analysis was that no peaks were found in the dextropropoxyphene area in the chromatograms. Two of the samples had small peaks in the area where nordextropropoxyphene usually elute but they were not identifiable and quantifiable according to the criteria of this method; retention time, relative response between fragment and pseudomolecular ion and LLOQ.

3.6. Method comparison with HPLC–UV

One of the most common ways to analyse dextropropoxyphene and its metabolite is HPLC with UV detection. This has been the method of choice at our laboratory and therefore a comparison between the methods could be undertaken. A number of patient samples ($n=20$ for dextropropoxyphene, $n=18$ for nordextropropoxyphene) were processed and analysed with both methods without any storage of the samples. The numerical data from the analyses were plotted against each other but only data that were within the standard curve were included in the

Table 4
Selectivity of the method

Sample no.	Analyte	Target (ng/ml)	Measured (ng/ml)	SD (ng/ml)	RSD (%)	Accuracy (%)
1–6	Dextropropoxyphene	500	558	10.0	1.8	11.6
	Nordextropropoxyphene	500	495	36.4	7.4	−1.0
7–12	Dextropropoxyphene	500	526	10.6	2.0	5.3
	Nordextropropoxyphene	500	548	16.2	3.0	9.6

Spiked compounds in sample 1–6: zolpidem, nefazodone, carbamazepine, alimemazine, dixyrazine and propiomazine. Spiked compounds in sample 7–12: zopiclone, flunitrazepam, levomepromazine, oxazepam, risperidone, 9-OH-risperidone.

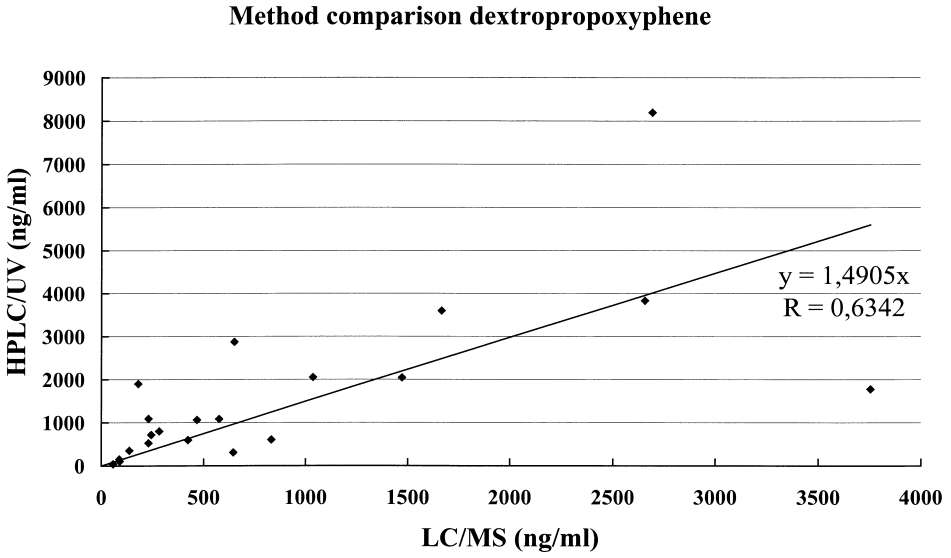


Fig. 2. Comparison between results from HPLC–UV and LC–MS for dextropropoxyphene.

comparison. The result from the comparison is shown in Figs. 2 and 3. The data from the LC–MS analyses is consistently lower than the data from HPLC–UV regarding dextropropoxyphene. A reason for this could be that the levels of dextropropoxyphene are often quite low and small peaks in UV chromatograms are more easily disturbed by other co-eluting substances than larger ones. This makes it often very difficult to separate them from each other and subsequently this means that the numerical data from HPLC–UV sometimes can be overestimates. On the other hand LC–MS is by far more selective and can be looked upon as a better method for dextropropoxyphene determinations in patient sam-

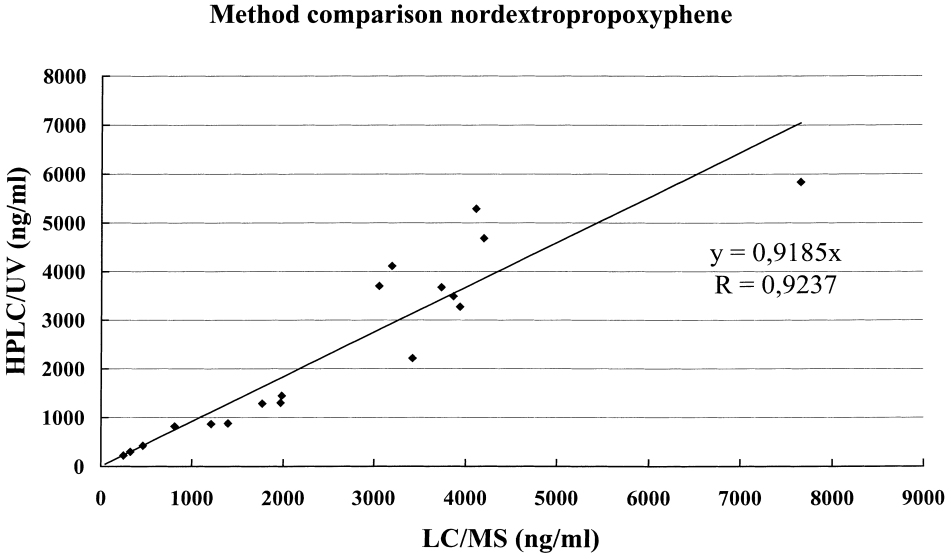


Fig. 3. Comparison between results from HPLC–UV and LC–MS for nordextropropoxyphene.

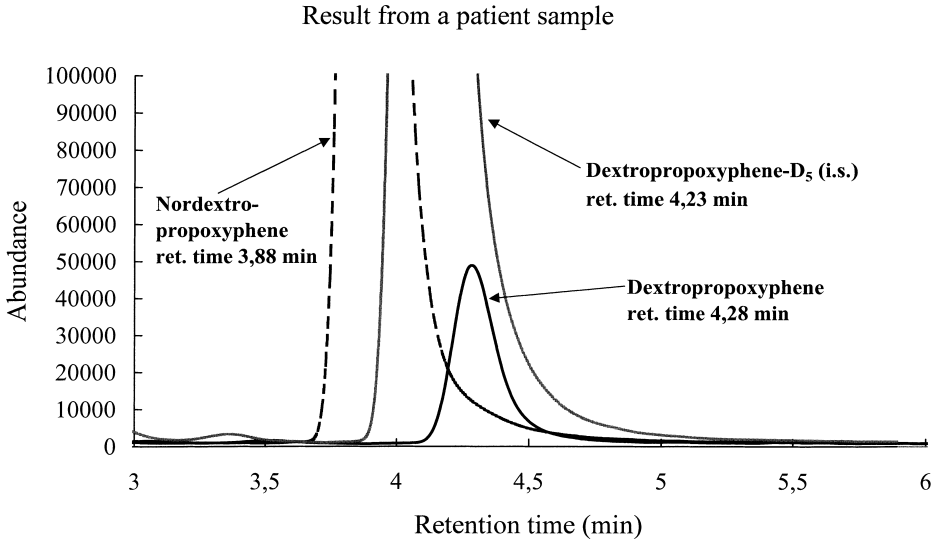


Fig. 4. Result from an analysis of a patient sample containing 235 ng/ml dextropropoxyphene and 3406 ng/ml nordextropropoxyphene.

ples. For nordextropropoxyphene the situation is somewhat different and the correlation between the two methods is much better. This is probably because the nordextropropoxyphene levels are higher and therefore the problems with co-eluting peaks in the UV chromatograms are not so grave. Fig. 4 shows an LC–MS chromatogram from a patient sample and Fig. 5 shows an LC–MS chromatogram of blank urine spiked with the internal standard dextropropoxyphene-D_5.

4. Conclusions

An LC–MS system consisting of a single quadrupole mass spectrometer was used to analyse the

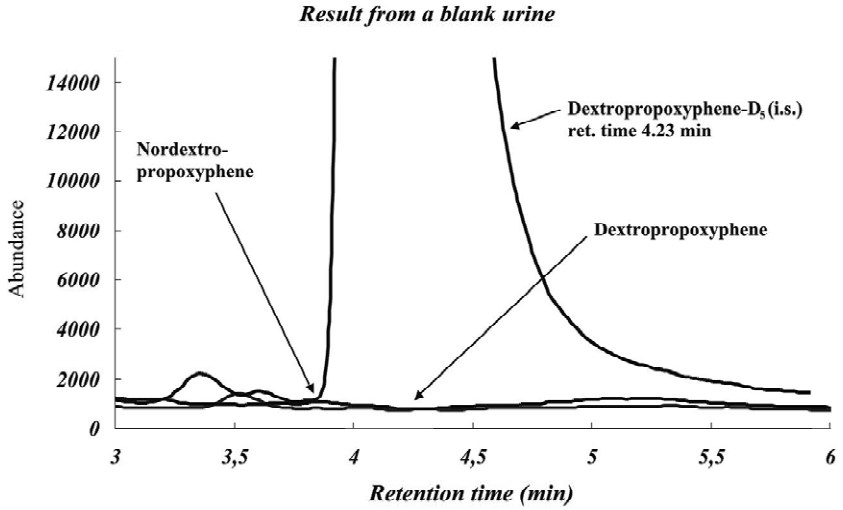


Fig. 5. Result from an analysis of blank urine spiked with the internal standard dextropropoxyphene-D_5. Retention time for dextropropoxyphene and nordextropropoxyphene are marked with arrows.

analgesic dextropropoxyphene and its main active metabolite nordextropropoxyphene. The analysis was performed in the selected ion-monitoring mode, where two ions per substance were monitored. To achieve fragmentation, in-source fragmentation was carried out and this produced one fragment plus the pseudomolecular ion $[(M+H)^+]$ per substance, which is enough to verify the sought compound in a sample. This is true only if the relative response between the two ions lies within a relatively small range and is reproducible. The identification criteria of the method is consequently chromatographic retention times along with the relative response between the chosen ions. A number of standard parameters were validated and samples from patients were analysed with both LC–MS and HPLC–UV. The comparison was not so excellent for dextropropoxyphene and this is probably because of the low levels in the samples and the problems of performing quantitation work with a non-selective detector like UV. The comparison was better for the metabolite nordextropropoxyphene were the levels are a number of times higher in real samples.

Finally, if only a decent work-up procedure and a good chromatographic separation is performed, problems with interference and ion suppression in the spray chamber of the mass spectrometer can be avoided. And thus a single quadrupole mass spectrometer can be utilised for the analysis of the sometimes-abused drug dextropropoxyphene.

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