

Development and Validation of ELISA and GC-MS Procedures for the Quantification of Dextromethorphan and its Main Metabolite Dextrorphan in Urine and Oral Fluid

Warren C. Rodrigues, Guohong Wang, Christine Moore*, Alpana Agrawal, Michael J. Vincent, and James R. Soares
Immunoanalysis Corporation, 829 Towne Center Drive, Pomona, California 91767

Abstract

The development of a highly sensitive enzyme-linked immunosorbent assay and gas chromatography-mass spectrometry confirmation method for the detection of dextromethorphan and its major metabolite dextrorphan in urine and oral fluid is described. For the screening assay, the intraday precision was less than 8% for urine and less than 5% for oral fluid. The interday precision was less than 10% for both drugs in urine and oral fluid. For the confirmatory procedure, both inter- and intraday precision was less than 5% for both matrices. The detection limit for both methods was 1 ng/mL. The quantifying ions chosen from the full scan mass spectra were m/z 271 for dextromethorphan, m/z 329 for dextrorphan, and m/z 332 for tri-deuterated dextrorphan- d_3 . A high recovery yield (> 93%) from the Quantisal™ oral fluid collection device was achieved, and the drugs were stable in the collection device for at least 10 days at room temperature. The extracted drugs from both matrices were stable for at least 48 h while kept at room temperature. Both screening and confirmatory procedures were applied to authentic urine and oral fluid specimens obtained from volunteers following therapeutic ingestion of dextromethorphan.

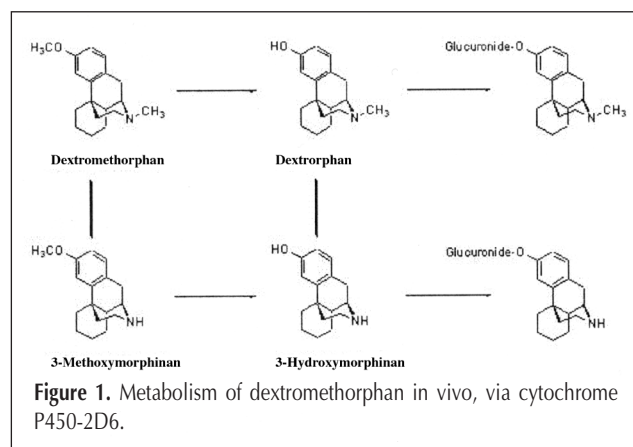
Introduction

Dextromethorphan (DXM) is an antitussive that is available in most over-the-counter cough medications and is being increasingly abused by teenagers across the United States. Dextromethorphan is a synthetic morphine analogue, first marketed in the 1970s. Dextromethorphan hydrobromide is the major ingredient found in a variety of non-prescription cough syrups (e.g., Robitussin®), tablets (e.g., Coricidin®), gel caps (e.g., Comtrex®), and lozenges (e.g., Sucrets®) (1). It is popularly known on the streets as “robo” and “skittles”, and its

illicit use, referred to as “robotripping”, is becoming popular among teenagers because of the ease of its availability, being a non-controlled substance. The California Poison Control System reported a 10-fold increase in DXM abuse cases from 1999 to 2004, with the median age of abusers being 16 (2). This makes it increasingly important to develop better analytical methods to easily and rapidly test for DXM usage as part of a teenage drug of abuse panel. Although urine analysis has been the preferred method of testing for drugs of abuse, oral fluid is increasingly useful matrix for drug testing because of its ease of observed collection and difficulty of adulteration.

Dextromethorphan is metabolized in the liver by cytochrome P450-2D6, into three metabolites: the major metabolite dextrorphan (DXO) and two minor metabolites 3-methoxymorphinan and 3-hydroxymorphinan. In addition, some is excreted as the conjugated glucuronides (Figure 1).

Both DXM and DXO act as potent blockers of the *N*-methyl-*d*-aspartate (NMDA) receptor. At high doses, the pharmacology of DXM is similar to that of the controlled substances phenylcyclidine (PCP) and ketamine. It has been found that approximately 10% of the population (depending on race) are poor metabolizers and hence are susceptible to greater toxicity due



* Author to whom correspondence should be addressed. E-mail: cmoore@immunoanalysis.com.

to ingestion of larger than normal doses of dextromethorphan (3). Also, people taking a number of antidepressants are more susceptible to DXM toxicity. Dextromethorphan typically has four plateaus of intoxication, ranging from mild euphoria from a 100–200-mg dose to full dissociation from a 500–1500-mg dose. Over-the-counter cold and cough medications also contain other active ingredients such as acetaminophen, chlorpheniramine, pseudoephedrine, and guaifenesin in addition to DXM, increasing the risks to an individual from DXM abuse because excess use can cause many symptoms associated with liver problems, severe nausea, or shortness of breath. These may be more extensively related to other drugs in the formulation rather than DXM itself.

Frèche et al. (4) have reported on the development of an enzyme linked immunosorbent assay (ELISA) involving separate assays for analysis of DXM and DXO in urine. Several groups (5–7) have focused on the development of liquid chromatography with tandem mass spectrometric detection (LC–MS–MS) methods for the identification of DXM and its metabolites; whereas other groups (8–11) have developed high-pressure liquid chromatographic procedures for the analysis of DXM and its metabolite in urine. Wu et al. (12) used capillary gas chromatography (GC) to analyze DXM and DXO in urine; and Salsali and co-workers (13) used GC with electron capture detection for analysis of dextrophan only. Finally, Kim et al. (14) developed a GC–MS procedure for detection of DXM and DXO in urine, following initial screening by thin-layer chromatography and then liquid–liquid extraction.

For the most part, toxicology laboratories have relied on PCP antibodies showing some degree of cross-reactivity with DXM, in order to screen for usage by ELISA. In this publication we report on the development of two methods for analysis of DXM and its metabolite. Part I of this paper focuses on the qualitative determination of DXM and its major metabolite DXO in human urine as well as oral fluid, using a competitive binding ELISA technique. Part II of this paper deals with a confirmation procedure using mixed mode solid-phase extraction, followed by electron impact GC–MS. The ELISA method is unique in the fact that it can detect both DXM and DXO in the same assay, and our GC–MS methodology allows for detection of both drugs in two separate matrices with the same extraction protocol.

Experimental

Materials

Reagents and chemicals

Unlabelled dextromethorphan and deuterated dextrophan were obtained from Cerilliant (Round Rock, TX). Dextrophan tartrate was purchased from Aldrich (Milwaukee, WI). 3,3',5,5'-Tetramethylbenzidine (TMB) substrate and the derivatizing reagent *N,O*-bis (trimethylsilyl) trifluoroacetamide + 1% trimethylchlorosilane (BSTFA + 1% TMCS) was obtained from Pierce (Rockford, IL). Ammonium hydroxide was purchased from Aldrich (Milwaukee, WI). All

solvents used were HPLC grade and from Spectrum Chemicals (Gardena, CA), and all chemicals were ACS grade. For ELISA, the high calibrators for DXM and DXO at 250 ng/mL were prepared in synthetic urine and oral fluid extraction buffer and stored at 4°C. For GC–MS, working solutions of calibrators at 1000 ng/mL in methanol and internal standard at 5000 ng/mL in methanol were prepared and stored at –20°C.

Apparatus

Quantisal devices for the collection of oral fluid samples were obtained from Immunalysis (Pomona, CA). They are equipped with collection pads with a volume adequacy indicator that allows for the collection of 1 mL of oral fluid (\pm 10%). The collection pads are then placed in the extraction buffer (3 mL) for transportation to the laboratory, thus diluting the oral fluid samples (1:3). This is accounted for in the final concentration of the analyzed oral fluid samples. Polystyrene microtiter plates (96 well) were obtained from Corning Costar (Corning, NY). Microtiter plate washer and reader were obtained from Tecan (San Jose, CA). Solid-phase extraction columns (Clin II, 691-0353T) were obtained from SPEWare (San Pedro, CA).

Methods

Part I: Enzyme-linked immunosorbent assay (ELISA)

A 96-well polystyrene microtiter plate was immobilized with DXM-specific IgG, obtained from immunization of rabbits with antigen consisting of DXM coupled to bovine thyroglobulin. The plate was stored in a sealed pouch with desiccant, as it is critical to keep it moisture free. DXM and DXO calibration curves were first prepared, by spiking negative synthetic urine at 1, 2.5, 5, 10, 25, 50, 100, and 250 ng/mL, from the high calibrator stock solutions of the drugs. A second set of calibration curves in oral fluid extraction buffer (diluted 1:4) at levels of 0.25, 1, 2.5, 5, 10, and 25 ng/mL was prepared, corresponding to neat oral fluid levels of 1, 4, 10, 20, 40, and 100 ng/mL. Urine specimens were pre-diluted 1:10 with 100mM phosphate buffered saline (pH 7.0) before analysis. Oral fluid specimens were analyzed directly from the Quantisal device. Calibrators and specimens were then pipetted in duplicate into the wells of the microtiter plate, using a 10- μ L sample size for urine or 50- μ L sample size for oral fluid, depending on the matrix being analyzed. This was followed by addition of 100 μ L of enzyme conjugate, consisting of DXM labeled with horseradish peroxidase (HRP). The plate was then allowed to incubate for 1 h in the dark, at room temperature. The wells were washed six times with 350 μ L of deionized water using a microtiter plate washer, then inverted and slapped dry to remove any residual moisture from the wells. TMB substrate (100 μ L) was then added to each well and the plate incubated for 30 min in the dark. The wells turned varying degrees of blue color depending on concentration. The reaction was then stopped with 100 μ L of 1N hydrochloric acid to produce a yellow color. The absorbance was read at a dual wavelength of 450 nm and 650 nm using a microtiter plate reader.

Part IIA: Sample preparation for GC analysis

An aliquot of urine (1 mL) or an aliquot from the Quantisal collection device (1 mL), equivalent to 0.25 mL neat oral fluid, was removed, and trideuterated DXO (10 μ L) was added as the internal standard at a concentration of 50 ng/mL. For each chromatographic analysis, calibration curves were prepared by spiking both negative urine and oral fluid at concentrations of 10, 25, 50, and 100 ng/mL, with DXM and DXO from the stock solutions of the drugs. Internal standard was also added to each calibration standard. Solid-phase mixed mode extraction columns (Clin II, 691-0353T) were placed into a positive pressure vacuum manifold. The columns were first conditioned with 2 mL methanol and 2 mL 0.1M phosphate buffer (pH 6.0). The urine or oral fluid specimens were then loaded onto the columns, and after flowing through, the columns were washed with 2 mL deionized water, 2 mL 0.1M acetate buffer (pH 4.0), 1 mL methanol, and 1 mL ethyl acetate. The columns were then allowed to dry under nitrogen pressure for about 3 min at 30 psi. The drugs were eluted from the columns with 2 mL freshly prepared ethyl acetate/ammonium hydroxide (98:2, v/v). The extracts were evaporated to dryness under nitrogen and reconstituted in ethyl acetate. Although DXM does not need derivatization for GC-MS analysis, DXO needs to be derivatized before injection into the GC-MS. The trimethylsilyl derivative of DXO was prepared by reconstituting the dried extracts in ethyl acetate (25 μ L) and BSTFA + 1% TMCS (25 μ L). The extracts were then transferred to autosampler vials, capped, and heated at 60°C for 20 min in a heating block.

Part IIB: GC-MS

An Agilent 6890N GC coupled to a 5975 mass selective detector (MSD) with an inert source, operating in electron im-

pact mode was used for analysis. The GC column was a DB-5MS (15-m length, 0.25-mm i.d., 0.25- μ m film thickness, J&W Scientific), and the injection temperature was 250°C. The purge flow rate was 50 mL/min for 1 min, and the carrier gas was helium. The injection mode was splitless, injection volume was 2 μ L, and the operation mode was constant flow at 1.5 mL/min. The initial oven temperature was 100°C, ramped at 40°C/min to 180°C, and then ramped at 5°C/min to 230°C. The transfer line was held at 280°C, the ion source at 230°C, and the quadrupole at 150°C. The dwell time for all ions was 50 ms. DXM was identified at a retention time of 6.6 min and three ions were selected from the full scan spectrum, m/z 271, 214 and 171. DXO was detected at a retention time of 7.22 min, and three ions were selected from the full scan spectrum, m/z 329, 314 and 150. DXO- d_3 was found at a retention time of 7.2 min, and the ions selected were m/z 332 and 153. The quantifying ions are shown underlined. Each analysis required the ion ratio between the quantifier ion and the two qualifier ions to be within $\pm 20\%$ in order to meet the criterion for a positive result.

Data analysis

Drug quantification of analytes was achieved with the Agilent DrugQuant ChemStation software package using deuterated DXO- d_3 as the internal standard. Calibration curves for both DXM and DXO were prepared from 10–100 ng/mL, using linear regression analysis and were forced through the origin. The mean correlation coefficient for urine was found to be $r^2 = 0.998$ (SD 0.0015) for DXM; $r^2 = 0.995$ (SD 0.004) for DXO. For oral fluid, the mean correlation was $r^2 = 0.997$ (SD 0.0025) for DXM and $r^2 = 0.995$ (SD 0.005) for DXO ($n = 3$). Full scan mass spectra for DXM and DXO are shown in Figure 2.

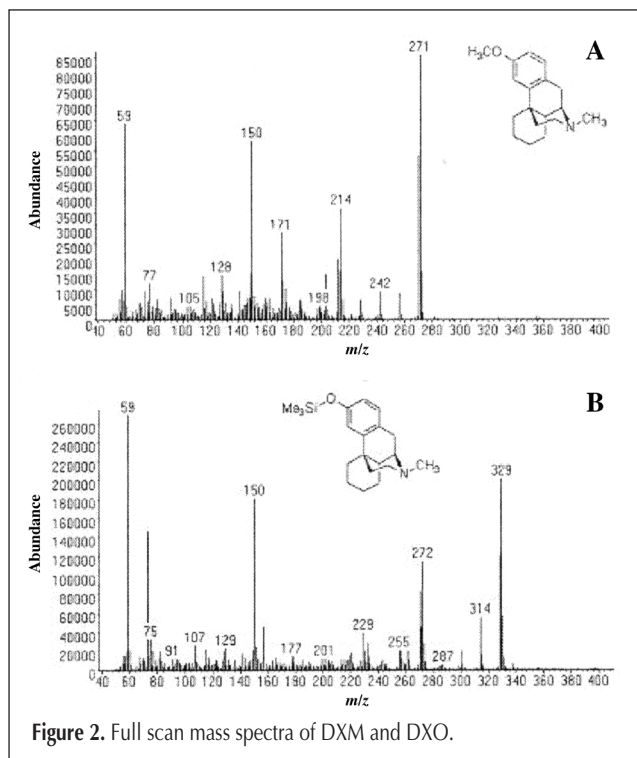


Figure 2. Full scan mass spectra of DXM and DXO.

Results and Discussion**Part I: ELISA****Dose-response curve**

Dose-response curves were prepared for both DXM and DXO in negative urine at levels of 1, 2.5, 5, 10, 25, 50, 100, and 250 ng/mL (Figure 3). A second set of dose response curves was prepared for both drugs in Quantisal extraction buffer

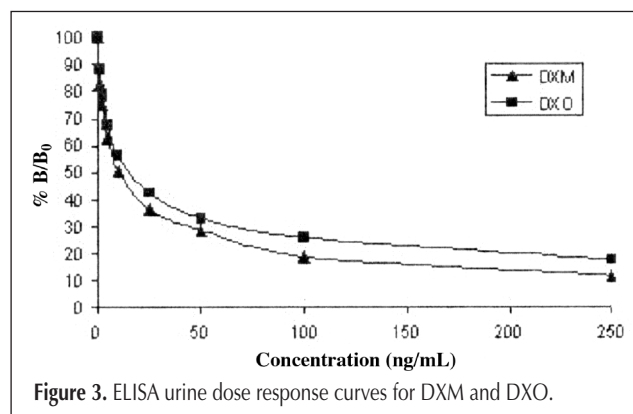


Figure 3. ELISA urine dose response curves for DXM and DXO.

(diluted 1:4) at levels of 0.25, 1, 2.5, 5, 10, and 25 ng/mL, corresponding to neat oral fluid levels of 1, 4, 10, 20, 40, and 100 ng/mL (Figure 4). The B/B_0 was calculated for each concentration level. B_0 is the absorbance of negative calibrator, and B is the absorbance of bound calibrator. The absorbance readings are inversely proportional to the concentration of drug in the sample. The reason being that the higher the drug concentration, the less drug-enzyme conjugate binds to the antibody, thereby producing a lower absorbance value. The HRP label is responsible for producing the characteristic blue color when TMB substrate is added to the wells in the microtiter plate.

Selectivity

Interference from related and non-related drugs was studied by spiking drug-free urine and oral fluid and performing the ELISA assay. Table I shows the cross-reactivity data with related drugs.

The following non-related drugs were analyzed at a concentration of 50,000 ng/mL: ethosuximide, methsuximide, normethsuximide, phenisuximide, mephenytoin, phenytoin, ethotoin, phenylethylmalonamide (PEMA), methyl PEMA, medazepam, oxazepam, lorazepam, diazepam, temazepam,

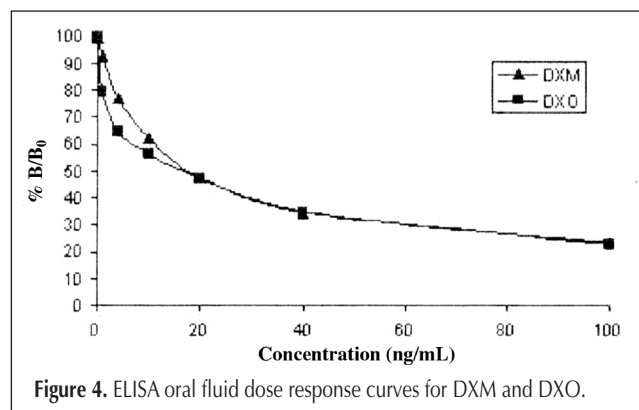


Figure 4. ELISA oral fluid dose response curves for DXM and DXO.

Table I. ELISA Cross-Reactivity with Related Drugs

Drug	Concentration (ng/mL)	% Cross-Reactivity
Levorphanol	50,000	ND*
PCP	5000	ND
Ketamine	5000	ND
LSD	5000	ND
Codeine	50,000	ND
Morphine	50,000	ND
Diacetylmorphine	50,000	ND
Ethyl morphine	50,000	ND
Hydrocodone	50,000	ND
Hydromorphone	50,000	ND
Meperidine	50,000	ND
Methadone	50,000	ND
Nalorphine	50,000	ND
Oxycodone	50,000	ND

* ND = not detected.

bromazepam, diazepam, flurazepam, amobarbital, butabarbital, hexobarbital, phenobarbital, secobarbital, barbital, methobarbital, mephobarbital, 10,11-dihydrocarbamazepine, carbamazepine, α -methyl- α -propylsuccinimide, 4-methyl-primidone, primidone, trimipramine, imipramine, desipramine, doxepin, nordoxepin, amitriptyline, nortriptyline, protriptyline, amphetamine, methamphetamine, methylenedioxymphetamine (MDA), methylenedioxyamphetamine (MDMA), methylenedioxyethylamphetamine (MDEA), caffeine, cocaine, benzoylecgonine, methylphenidate, propoxyphene, diphenhydramine, glutethimide, methprylon, meprobamate, chlorpromazine, methaqualone, lidocaine, methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), ephedrine, phentermine, Δ^9 -THC, 9-carboxy THC, fluoxetine, sertraline, zolpidem, buprenorphine, cotinine and acetaminophen. None of these drugs were found to interfere with the detection of DXM and DXO. Levorphanol a narcotic analgesic is the levo isomer of dextrophan, found in medications like Levo-Dromoran[®]. The antibody is highly specific to the dextro isomer only and hence shows no cross-reactivity with levorphanol.

Precision

ELISA intraday and interday precision was determined for

Table II. ELISA Intraday and Interday Precision for DXM and DXO in Urine

Drug	Intraday (n = 8) (CV %)	Interday (n = 40) (CV %)
Dextromethorphan		
10 ng	6.75	4.34
25 ng	7.25	6.37
50 ng	5.63	8.34
Dextrorphan		
10 ng	3.41	9.04
25 ng	4.91	8.83
50 ng	5.06	9.91

Table III. ELISA Intraday and Interday Precision for DXM and DXO in Oral Fluid

Drug	Intraday (n = 8) (CV %)	Interday (n = 40) (CV %)
Dextromethorphan		
1 ng	2.73	5.43
4 ng	1.94	5.56
10 ng	2.86	5.04
20 ng	3.13	7.35
Dextrorphan		
1 ng	3.41	7.61
4 ng	4.58	8.45
10 ng	3.89	6.89
20 ng	3.72	6.13

both matrices. Intraday precision was calculated from eight analyses carried out on the same day ($n = 8$), and interday precision from eight analyses per day, over five days ($n = 40$), shown in Tables II and III.

Part II: GC-MS

Selectivity

Drug-free urine and oral fluid samples were extracted and analyzed exactly as outlined in our procedure in order to de-

Table IV. GC-MS Intraday and Interday precision for DXM and DXO in Urine

Drug	Expected Concentration (ng/mL)	Observed Concentration (mean \pm SD) (ng/mL)	Precision (CV%)
Intraday ($n = 5$)			
Dextromethorphan	25	24.97 \pm 0.54	2.16
Dextrorphan	25	26.61 \pm 0.85	3.19
Interday ($n = 10$)			
Dextromethorphan	25	25.00 \pm 0.90	3.60
Dextrorphan	25	26.68 \pm 1.23	4.61

Table V. GC-MS Intraday and Interday Precision for DXM and DXO in Oral Fluid

Drug	Expected Concentration (ng/mL)	Observed Concentration (mean \pm SD) (ng/mL)	Precision (CV%)
Intraday ($n = 5$)			
Dextromethorphan	25	27.38 \pm 1.14	4.16
Dextrorphan	25	25.02 \pm 1.20	4.79
Interday ($n = 10$)			
Dextromethorphan	25	29.06 \pm 1.40	4.82
Dextrorphan	25	27.11 \pm 1.26	4.65

Table VI. Extraction Efficiency of DXM and DXO from the Quantisal Collection Device, Overnight at RT ($n = 6$)

Quantisal Device	Dextromethorphan (ng/mL)	Dextrorphan (ng/mL)
No pad	26.36	25.84
With pad 1	27.52	25.92
2	23.44	23.88
3	23.00	24.24
4	22.72	22.72
5	23.84	23.88
6	26.84	25.00
Mean	24.56	24.27
SD	1.89	0.99
CV (%)	7.69	4.08
Drug recovery (%)	93.1	93.9

termine interference from both these matrices on the GC-MS assay. Spiking negative urine and oral fluid and then subjecting them to extraction and GC-MS analysis as previously described also allowed interference from related and non-related drugs to be studied. The same drugs described earlier were added at concentrations of 50,000 ng/mL. None of these drugs were detected by the GC-MS method and hence showed no interference with the analysis. Levorphanol is the only drug that was detected by this GC-MS method. This is to be expected, because GC-MS does not discriminate between the dextro and levo isomers of a compound without chiral analysis. This is not, however, presumed to be an issue because the preliminary ELISA screen would eliminate levorphanol, excluding the possibility of a false-positive result by GC-MS.

Linearity and sensitivity

GC-MS calibration curves for both DXM and DXO were found to be linear from 0 to 100 ng/mL in both urine as well as oral fluid. The sensitivity of the method was determined by establishing the limit of quantitation (LOQ) defined as the lowest concentration detectable with a signal-to-noise (S/N) ratio of at least 10 and retention time within 0.2 min of the calibration standard. The limit of detection (LOD) was determined from

Table VII. Stability of Extracted Urine Samples at RT ($n = 3$)

Replicate	24 h		48 h	
	DXM (ng/mL)	DXO (ng/mL)	DXM (ng/mL)	DXO (ng/mL)
1	23.56	24.54	24.50	25.05
2	23.83	25.69	24.72	26.12
3	23.38	24.82	24.16	25.21

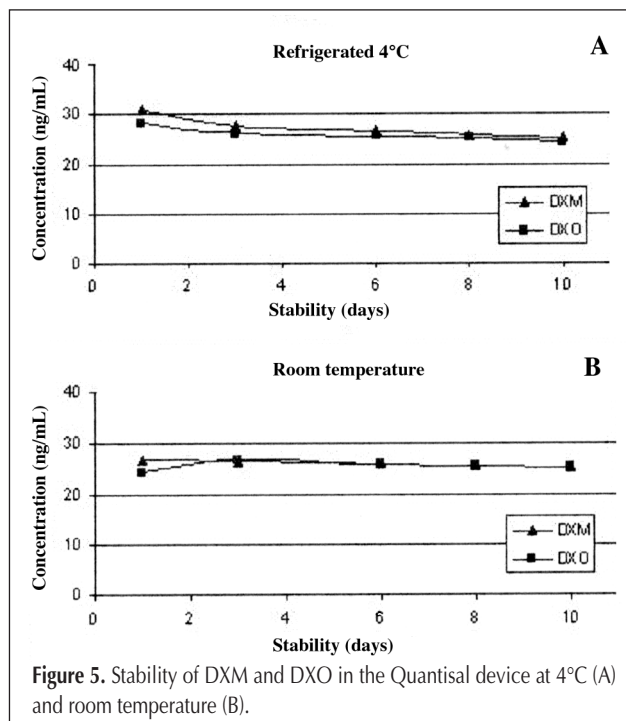


Figure 5. Stability of DXM and DXO in the Quantisal device at 4°C (A) and room temperature (B).

the lowest concentration detectable with an S/N ratio of at least 3. The limit of detection was found to be 1 ng/mL for both drugs, and the limit of quantitation was 10 ng/mL in both matrices.

Precision

Intraday precision of the method was determined from five analyses performed on the same day for both urine and oral fluid matrices at the calibration level of 25 ng/mL and was found to be < 5%. Interday precision was established from 10 analyses of urine and oral fluid samples at the calibration level of 25 ng/mL, performed over a period of 5 days (2 per day) and was < 5%. Precision was defined as the standard deviation expressed as the percentage coefficient of variation. Data are

Table VIII. Stability of Extracted Oral Fluid Samples at RT (n = 3)

Replicate	24 h		48 h	
	DXM (ng/mL)	DXO (ng/mL)	DXM (ng/mL)	DXO (ng/mL)
1	26.02	24.82	26.52	25.13
2	27.81	25.49	28.45	25.77
3	26.62	24.67	27.34	24.98

Table IX. ELISA and GC-MS Results of Authentic Urine Specimens

Time (h)	Subject # 1			Subject # 2			Subject # 3		
	ELISA*	GC-MS (ng/mL)		ELISA	GC-MS (ng/mL)		ELISA	GC-MS (ng/mL)	
Pre-dose	N	0	0	N	0	0	N	0	0
3	P	225	2918	P	51	536	P	705	2039
6	P	24	226	P	9.9	106	P	803	1073
12	P	13	155	P	3.4	26	P	98.6	279
24	P	2.2	31.5	P	5.7	30	-	-	-
48	N	2.8	4.7	N	2.5	3.8	-	-	-

* P = B/B₀ ≤ 40%; N = B/B₀ > 40% (cut-off = 25 ng/mL).

Table X. ELISA and GC-MS Results of Authentic Oral Fluid Specimens

Time (h)	Subject # 1			Subject # 2			Subject # 3		
	ELISA*	GC-MS (ng/mL)		ELISA	GC-MS (ng/mL)		ELISA	GC-MS (ng/mL)	
Pre-dose	N	0	0	N	0	0	N	0	0
3	P	5.5	7.8	P	5.7	4.9	P	76.6	10.9
6	P	4.7	4.7	P	2.8	3.1	P	74.8	11.3
12	P	1.6	2.0	N	1.5	1.8	P	58	9.7
24	N	1.3	1.4	N	1.5	1.6	-	-	-
48	N	0.9	1.4	N	0.9	1.4	-	-	-

* P = B/B₀ ≤ 70%; N = B/B₀ > 70% (cut-off = 4 ng/mL).

shown in Tables IV and V.

Extraction efficiency of Quantisal device

Extraction efficiency from the pad in the Quantisal device was determined by fortifying synthetic oral fluid with DXM and DXO at the calibration level of 25 ng/mL and then placing the pad in the fluid until the volume adequacy indicator turned blue, showing that 1 mL oral fluid (± 10%) had been absorbed. The pads were then placed in the buffer contained in the collection device and stored overnight at room temperature to simulate transport to a laboratory. The next day the pads were removed with serum separators, and a 1-mL aliquot was extracted and analyzed by the GC-MS procedure outlined earlier. This procedure was repeated six times for verification, giving an overall extraction efficiency for both drugs from the pad of > 90%. The results are shown in Table VI.

Stability

Urine. In the case of the urine, the stability of the extracted samples was examined. The autosampler vials containing the extracts were stored at room temperature for 24 h and 48 h and then re-analyzed and compared to the analysis from the first day (Table VII).

Oral fluid. In order to determine the stability of the drugs in the Quantisal collection devices, DXM and DXO were spiked in synthetic oral fluid at the 25 ng/mL calibration level and then one set were stored at room temperature and another in the refrigerator (4°C) for 10 days. Aliquots (1 mL) were then extracted and analyzed by GC-MS after 1, 3, 6, 8, and 10 days (Figure 5). The stability of the extracted samples was also determined (Table VIII).

Authentic specimens

A time-dependent dosage study for DXM was performed using three volunteers from our laboratory, in order to assess the effectiveness of our ELISA and GC-MS methodology. Volunteers ingested a therapeutic or twice therapeutic dose of DXM gel caps (30 mg), following which oral fluid samples using the Quantisal device as well as urine samples were collected after intervals of 3, 6, 12, 24, and 48 h. The specimens were first screened using ELISA. All samples were then subjected to solid-phase extraction and analyzed by GC-MS using the described procedures. The results are shown in Tables IX and X. Urine samples were diluted 1:10 with 100mM PBS pH 7.0 before analysis, so as to fall within the calibration curve range. Alternatively for urine specimens, the sample volume may be reduced to 100 mL for solid-phase extraction, and then the appropriate multiplication factor applied to correct for this during GC-MS quantification. Oral fluid specimens were used directly without dilution from the Quantisal device, but multiplied by a factor of 4 during quantification, to compen-

sate for the dilution of neat oral fluid in the oral fluid extraction buffer. Of the 16 samples tested, 7 tested positive using both ELISA and GC-MS, 8 were negative by both assays, and 1 was negative using GC-MS but screened positive using ELISA.

In the case of urine samples, we observed a higher ratio of DXO/DXM due to metabolism in the liver with a peak ratio occurring after roughly 3 h after intake of the drug. It should be noted that although the total DXM and DXO levels in most of the oral fluid specimens and some urine specimens appear to be rather low, this should typically not be the case with an abuser's oral fluid or urine specimens. Of the 16 urine samples tested, 11 tested positive using both ELISA and GC-MS, and five were negative by both assays.

Conclusions

This paper describes robust methods for the analysis of dextromethorphan and its metabolite dextrorphan in urine and oral fluid by ELISA and GC-MS. Both techniques give good precision, specificity and sensitivity. A comparison of the two shows them to be highly accurate for both testing matrices. Oral fluid has become a useful testing matrix, due to ease of observed collection and difficulty of adulteration. The drugs are quite stable in the oral fluid extraction buffer and the recovery of both the drugs from the Quantisal device is relatively high, making oral fluid viable for determining DXM abuse. These methods would be extremely useful, as they can be applied to the determination of dextromethorphan in the forensic toxicology field as part of a teenage drug of abuse test panel.

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