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QUANTITATION OF DRUGS OF ABUSE BY GC/MS. THE EUROPEAN EXPERIENCE

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INTRODUCTION

A two steps survey involving twelve Member States of the European Union was performed in 1993 and 1994 under the sponsorship of the DG V/F/1 of the Commission of the European Union in order 'to examine the reliability of urinalyses carried out to detect the use of illicit drugs'.

The design of the survey tried to gain insight into the following main issues:

- Analytical methodologies more often used in drugs of abuse testing in the EU
- Analytical strategies and toxicological criteria applied for the analysis and evaluation of results
- Final results released from the laboratories after the application of their analytical strategy and toxicological criteria.

MATERIAL AND METHODS

Materials

A set of six sterile spiked urine samples of unknown content was distributed among 195 participant laboratories together with a comprehensive data collection form (survey part I). The laboratories had to analyze the content of the sample (unknown to them) and report the results. In addition to data on preliminary screening and confirmation, laboratories had the possibility of reporting quantitative results of the drugs. Laboratories were requested to report the analytical methods used in the data collection form.

In the second step of the survey (part II) spiked urines with different mixtures of the same drugs but at similar concentrations to the first step were sent to laboratories and also clinical urines (obtained from drug addicts) were introduced for their analysis. As much as 228 laboratories were involved in the second step including a group of 154 laboratories which had participated in the first survey step.

Groups of drugs included were: amphetamines, opiates (including methadone), cocaine and cannabinoids. Specific substances to identify and quantify (if procedures available) were: amphetamine, methamphetamine, morphine (free and conjugated), codeine (free and conjugated), 6-acetylmorphine, methadone, EDDP (only in the

second step of the survey), benzoylecgonine, ecgonine methyl ester and 11nor-9COOH-Δ^9-THC. See Table 1 for concentrations of substances tested.

In the second step of the survey freeze-dried urine samples and drug standards solutions were distributed to each participating laboratory with indication of their contents in order to optimize the reliability of analytical methods. Additionally deuterium labelled drugs were distributed exclusively to those repeater laboratories that had performed quantification by GC/MS in the first step of the survey (31 laboratories).

Table 1.- Substances tested. Concentrations reported for A and B are theoretical values based on the gravimetric addition of constituents into the drug free urine. Concentrations reported for C are the mean of quantitative results obtained by reference laboratories (excluding the higher and the lower value for each parameter) in clinical samples. Concentrations units: ng/mL.

Group of drugs	Substance	A Drugs common in both surveys (b)	B Additional drugs in Survey Part I (a)	C Additional drugs in Survey Part II (b)
Amphetamines	Amphetamine	1000		
	Amphetamine Methamphetamine	300 1000		
Opiates	Total morphine Total codeine 6-acetylmorphine	1680 200 500		5183 249 48
	Total morphine Total codeine	370 600		361 1493
Methadone	Methadone EDDP		1000	1318 2669
	Methadone EDDP		500	470 638
Cocaine	Benzoylecgonine Ecgonine methyl ester		1000 600	3029 521
	Benzoylecgonine Ecgonine methyl ester	400 200		
Cannabinoids	11nor-9COOH-Δ^9-THC		31(b)	28
	11nor-9COOH-Δ^9-THC		180 (racemate)	120

(a) spiked samples (b) clinical samples

Methods

Samples validation content was done by a group of reference laboratories.

In order to evaluate quantitative results for each specific substance and each concentration, the following parameters were considered:

Outliers: results being above (>2 times) or below (<0.5 times) the true value (mean of reference laboratories) expressed as percentage. Given the toxicological aspects of drugs of abuse testing, this easily operative definition was preferred over others based on statistical terms.

Inaccuracy: Absolute differences between the mean of laboratories (outliers excluded) and the true value, divided by the true value expressed as percentage.

Lack of precision: coefficient of variation (outliers excluded).

In order to evaluate quantitative results for each analytical method, the following parameter was considered:

Inaccuracy: Absolute differences between the mean of laboratories and the true value, divided by the true value expressed as percentage.

Lack of precision: coefficient of variation.

RESULTS

Analysis performed

The quantitative analytical approach for specific substances was performed by a relatively low percentage of laboratories. Rates of analyses performed in the first and the second step of the survey are presented in Figure 1. There is an increase in the number of quantitative analyses reported by laboratories in the second step of the survey, specially important for 6-acetylmorphine and ecgonine methyl ester. The rate of quantitative analyses performed in survey part I and in survey part II was 18% and 25.6% respectively.

Analytical methods used

GC/MS was the leading technique in use for quantification and accounts for a 58% of all quantifications performed in survey part I. GC/MS was even more oftenly used in the second step of the survey (63% of all quantifications performed). GC ranked as the second method more often used (16% of analyses in both steps of the survey) followed by HPLC (10% in survey part I and 13% in survey part II). Lower rates of quantitative results were reported using other chromatographic methods including conventional TLC, HPLC/MS and GC/FTIR. A relevant number of quantifications were performed applying immunoassays especially in survey Part I (16% of all quantifications performed).

Results for specific substances at different concentration levels

Table 2 shows results obtained by laboratories in the second step of the survey when evaluating specific substances at different concentration levels following the parameters previously defined.

Table 2.- Results obtained by laboratories in survey Part II when evaluating specific substances at different concentration levels in spiked (S) and clinical (C) samples. Substances are ranked from the best to the worst performance according to the degree of difficulty found by laboratories taking into account results obtained for the three parameters evaluated.

Substance		conc. (ng/mL)	OUTLIERS (%)	LACK OF PRECISION (cv%)	INACCURACY (%)
9COOH-delta9-THC	S	120	3.4	23.7	2.7
Codeine	S	600	9.6	19.0	5.4
6-Acetylmorphine	S	500	10.5	23.8	4.7
Methamphetamine	S	1000	16.4	25.5	2.5
Benzoylecgonine	S	400	12.9	23.2	6.3
Methadone	C	470	4.5	24.9	13.1
Morphine	S	1680	11.1	29.0	6.0
Methadone	C	1318	15.6	26.1	6.5
Codeine	S	200	12.8	23.5	19.5
EDDP	C	2669	22.2	26.8	3.9
6-Acetylmorphine	C	48	19.4	27.5	6.5
Benzoylecgonine	C	3029	16.9	26.0	13.6
Morphine	C	361	25.0	34.8	3.5
Morphine	S	370	27.5	25.9	8.1
Amphetamine	S	1000	12.3	30.3	12.8
EME	S	200	26.5	23.8	15.5
Morphine	C	5183	30.9	30.7	3.9
EDDP	C	638	21.7	28.9	9.6
Amphetamine	S	300	23.1	27.3	11.9
EME	C	521	21.2	26.2	26.0
Codeine	C	1493	50.0	42.6	3.0
9COOH-delta9-THC	C	28	37.5	35.3	10.1
Codeine	C	249	48.8	43.6	8.0

The highest number of outliers and the largest coefficients of variation were associated with the quantification of codeine in clinical samples, probably due to large variations in the efficiency of the hydrolysis step in sample preparation before its submision to chromatographic analysis. A similar explanation can be apllied to the poor performance observed in the quantification of 11nor-9COOH-Δ^9-THC in clinical samples and total morphine in samples containing morphine-3-O-glucoronide (for both clinical and spiked samples). Rates of outlier results and coefficients of variation observed in spiked samples and in clinical samples are shown in Figure 2. It is apparent a better performance when analyzing spiked samples rather than the clinical ones.

Results obtained by the group of 31 repeater laboratories that performed quantification by GC/MS in the first step of the survey and that were provided with deuterated labelled drugs in the second part of the survey are shown in Table 3. Only substances spiked at the same concentrations in the two surveys were evaluated. A general improvement of reported results is observed for all substances except for benzoylecgonine at low concentrations. No outlier results have been reported for amphetamine and codeine, both at high concentrations and for methamphetamine. Coefficients of variations for each analyte decreased considerably after the provision of deuterated drug standards for all parametrers except for 6-acetylmorphine.

Table 3.- Results obtained by GC/MS by repeater laboratories provided with deuterated labelled drugs in survey Part II, for those concentrations common to the two survey's steps.

Substance	conc. (ng/mL)	OUTLIERS (%)		LACK OF PRECISION (CV)	
		Survey Part I	Survey Part II	Survey Part I	Survey Part II
Amphetamine	1000	182	0	311	252
Amphetamine	300	200	77	377	255
Methamphetamine	1000	227	0	391	218
Morphine	1656	296	125	401	319
Morphine	363	423	217	455	296
Codeine	600	111	0	279	149
Codeine	200	120	42	375	222
6-acetylmorphine	500	250	77	220	237
Benzoylecgonine	400	136	185	323	198
Ecgonine methyl ester	200	333	200	32.6	222

The mean of quantitative results obtained by repeater laboratories for each parameter

does not vary substantially from theoretical values derived from concentrations used for sample spiking.
In Figure 3 global rates of outliers results and coefficients of variations obtained by laboratories are shown. An improvement of quantitative results reported by the group of laboratories evaluated after the provision of analog deuterated drug standards is observed when comparing both survey steps.

Results for each analytical method

Table 4 shows the mean and coefficients of variations of quantitative results reported in survey part II for each chromatographic analytical method when quantifying specific substances at different concentrations. In general terms the lowest coefficient of variation (excluding opiates results) has been observed when using GC/MS while the highest coefficient of variation corresponds to HPLC results (see Figure 4).

Table 4.- Inaccuracy and lack of precison of results obtained by laboratories using chromatographic methods when quantifying specific subtances (outlier values not excluded).

Substance	conc. range ng/ml	HPLC		GC		GC/MS	
		Inac. (%)[a]	c.v. (%)[b]	Inac. (%)	c.v. (%)	Inac. (%)	c.v. (%)
Amphetamine	286-971	817.9	163.3	49.9	55.3	24.6	54.6
Methamphetamine	1077	56.4	93.3	26.2	57.7	5.0	69.9
Total morphine	293-5183	21.8	64.0	69.1	53.2	12.0	60.5
Total codeine	178-1493	12.3	52.1	137.2	76.4	25.3	65.3
6-acetylmorphine	48-413	7.6	21.9	314.0	90.9	40.1	98.0
Methadone	470-1318	37.7	51.7	7.9	32.9	7.6	30.4
EDDP	638-2669	15.4	68.2	21.2	42.9	6.7	42.6
Benzoylecgonine	317-3029	14.9	56.4	59.0	96.9	12.7	46.4
Ecgonine methyl ester	135-521	[c]		33.6		30.4	57.7
11nor-9COOH-delta9-THC	28-101	1305.0	138.1	74.2	34.3	16.3	37.2

a) inac.=innacuracy, b) cv=lack of precision, c) no data available

Table 5 shows results obtained by laboratories using GC/MS for quantification. Results obtained by those laboratories where deuterium labelled ISTD were provided (31 laboratories) are compared with the rest of laboratories using GC/MS.

The mean of coefficients of variations (excluding opiates) in both groups shows that the use of analog deuterated drugs as internal standards improves drugs of abuse

quantification by GC/MS (Figure 5).

Table 5.- Precision and accuracy of quantitative results obtained by laboratories provided with deuterium labelled substances versus the rest of laboratories using GC/MS.

Substance	conc. range ng/ml	no deuterated ISTD		ISTD deuterated	
		inaccuracy	lack of precision c.v.%	inaccuracy	lack of precision c.v.%
Amphetamine	286-971	43.9	64.1	8.4	225
Methamphetamine	1077	62.8	71.5	8.3	23.4
Total morphine	293-5183	13.0	37.8	18.9	63.8
Total codeine	178-1493	30.9	67.6	24.3	53.3
6-Acetylmorphine	48-413	40	24.9	67.8	978
Methadone	470-1318	4.3	29.4	14.6	28.3
EDDP	638-2669	9.6	53.6	5.5	35.7
Benzoylecgonine	317-3029	10.5	46.4	13.3	39.6
Ecgonine methyl ester	135-521	58.2	66.3	17.2	44.3
11nor-9COOH-delta9-THC	28-101	20.8	40.7	14.0	33.8

CONCLUSIONS

The quantitative analytical approach for specific substances is performed only by a relatively low percentage of laboratories. The availability of reference substances helps laboratories in the setting up of quantitative analytical methods.

A poor performance in quantitative results reported is observed. Specifically there are important problems in the quantification of opiates and cannabinoids probably because large variations in the yields of the hydrolysis step in the sample preparation procedure (especialliy for opiates). Nevertheless, it should be notice also a general improvement in quantitative results observed in the second part of the survey due to the provisison of reference substances and freeze dried reference urines and also because the past experience in survey part I.

Quantitative results obtained by laboratories in survey part II for spiked samples, are better than those obtained in clinical samples.

Results obtained by GC/MS (indepently of the use of analog deuterated internal standards) are better than those obtained by other chromatographic techniques (i.e. HPLC, GC).

The group of 31 repeater laboratories that were provided with analog deuterated internal standards in the survey Part II for GC/MS had a better performance when comparing results of substances with the same concentrations common to both surveys (13% reduction of outlier results, 11% reduction of coefficient of variation).

Laboratories quantifying by GC/MS and using analog deuterated internal standards as compared with those using GC/MS without analog deuterated internal standards) have lower coefficients of variation.

GC/MS has the best performance for drugs of abuse quantification. The use of analog deuterated standards should be strongly encouraged. Nevertheless, efforts should be continued to further improve the reliability of quantitative results for drugs of abuse urinalysis.

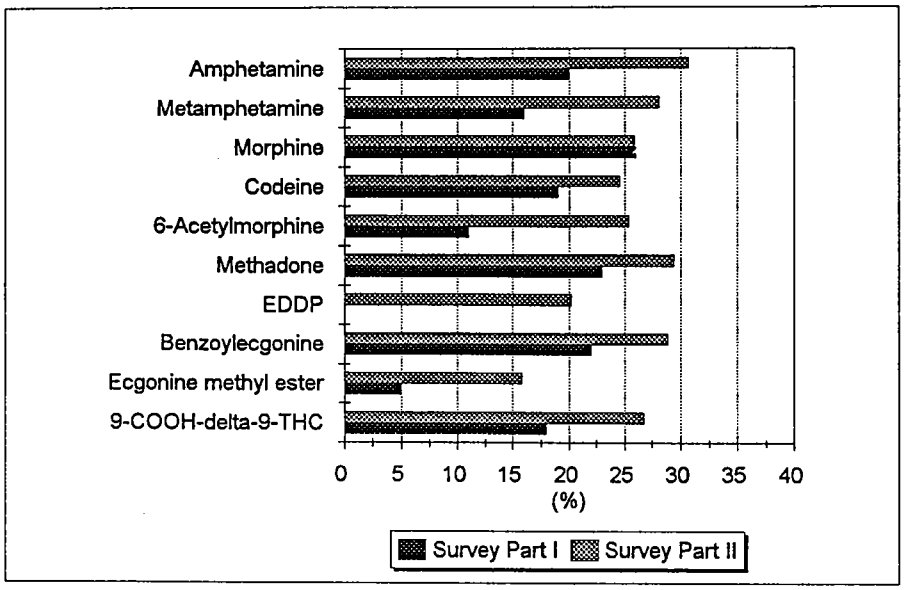

Figure 1.- Rate of quantitative results reported by substance

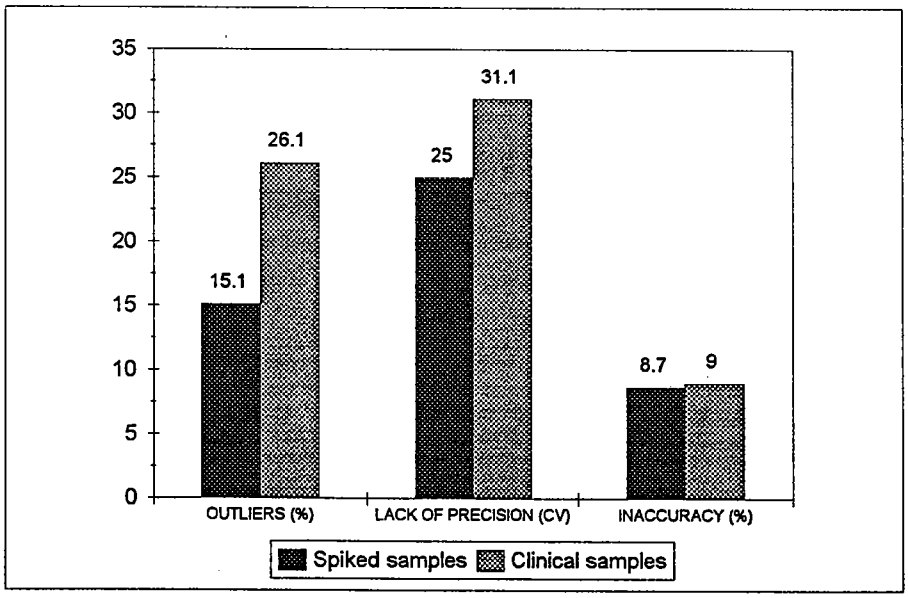

Figure 2.- Variability in quantitative results for drugs of abuse according the type of sample analyzed

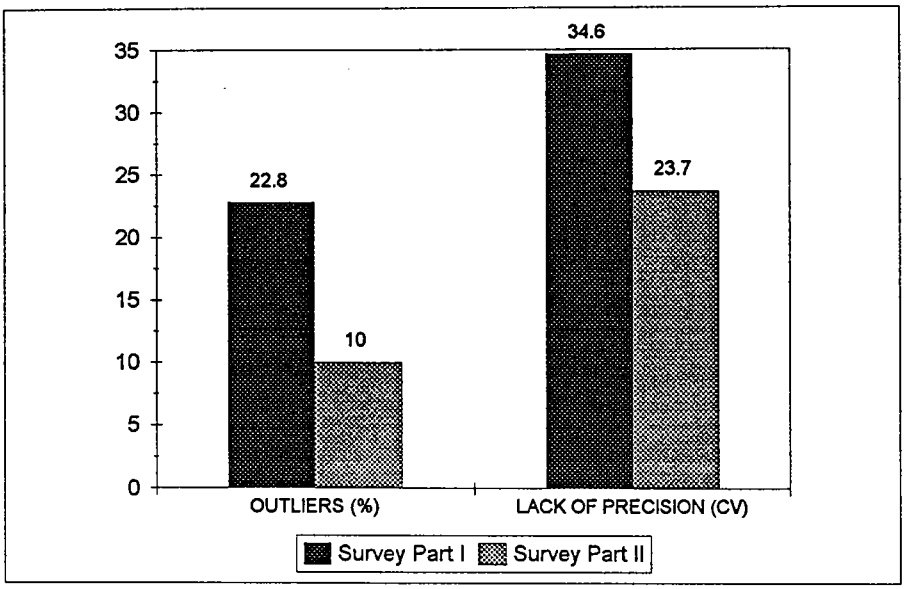

Figure 3.- Rates of outlier results and variability of quantitative results reported by laboratories using GC/MS and provided with deuterated ISTD

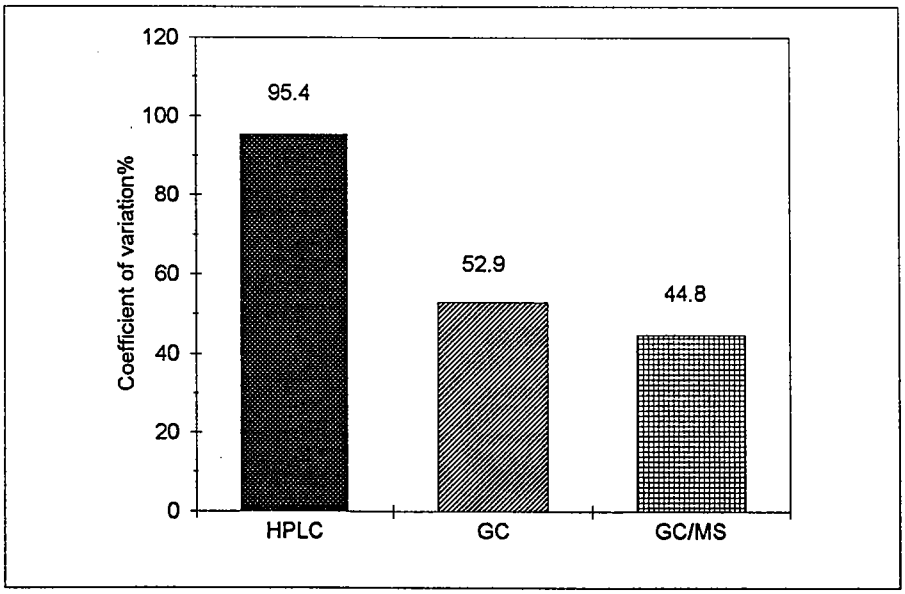

Figure 4.- Coefficients of variation observed by technique

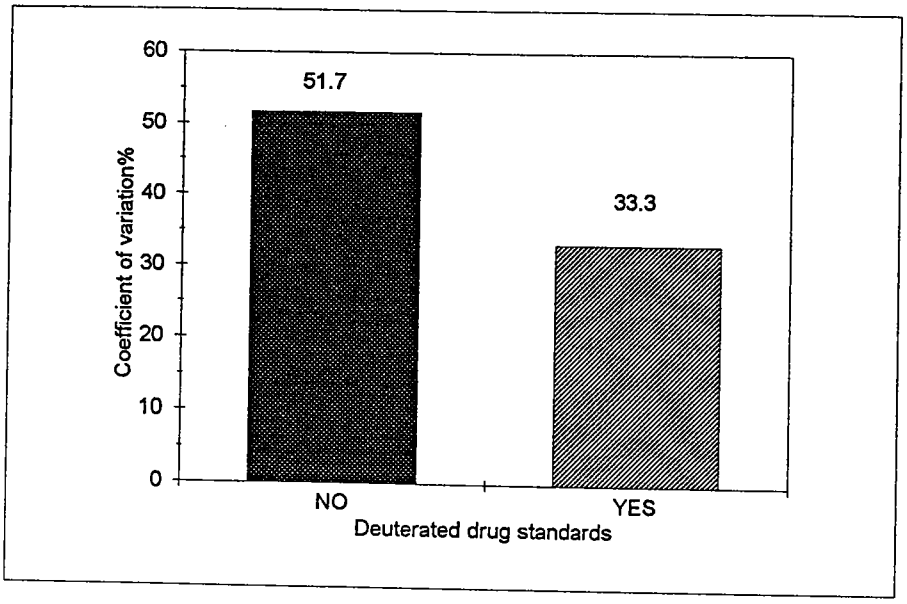

Figure 5.- Coefficients of variation observed in those laboratories applying GC/MS and using/or not analog deuterated internal standards for quantification