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HORIZONTAL STANDARDS ON ORGANIC MICRO-POLLUTANTS FOR IMPLEMENTATION OF EU DIRECTIVES ON SLUDGE, SOIL AND TREATED BIO-WASTE

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DEVELOPMENT OF AN ANALYTICAL METHOD FOR PHARMACEUTICAL PRODUCTS IN SLUDGE, SOILS AND SEDIMENTS

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List of Abbreviations

- Beza : Bezafibrate
- Carba : Carbamazepine
- Diclo : Diclofenac
- Diclo-d_4 : Diclofenac-d_4
- Gem : Gemfibrozil
- Gem-d_6 : Gemfibrozil d_6
- Ibu : Ibuprofen
- Keto : Ketoprofen
- LOQ : Limit Of Quantification
- Meto : Metoprolol
- Napro : Naproxen
- Para : Paracetamol
- Para d_4 : Paracetamol d_4
- Phena : Phenazone
- Phena-d_3 : Phenazone-d_3
- Primi : Primidone
- Propra : Propranolol
- Propra-d_7 : Propranolol-d_7
- RSD : Relative Standard Deviation
- US : Ultra Sonication

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FOREWORD

The European project Horizontal is focussed on the standardization of test procedures in environmental samples. Several studies have been started to elaborate the possibility of horizontal standardization on specific subjects. One of the subjects is the Pharmaceuticals Products. The aim of this project is to build a standard or a report close to a standard but the final step of this particular project ends at the second consultation. The validation and transfer to Task Force 151 will not carry out.

We present here the final report on the development of an analytical method to analyse the pharmaceutical products in solid matrices.

INTRODUCTION

Since their identification in water, pharmaceutical compounds have been targeted as emerging environmental contaminants. Their chemical properties (Log Kow, pKa, polarity….) show tendency towards persistence in solid environmental matrices.

Due to their polarity, persistence and water solubility, some drugs and metabolites are able to pass through the wastewater treatment plants. Their low adsorption on sludge and soils may cause the contamination of surface and ground water.
The sorption on sludge or soils could let original active substance in hydrophobic links persistent. For some of them, partial or total biodegradation may occur possibly producing unknown metabolites more or less active than initial form.

1. SCOPE

The purpose of this report is to present the development of an analytical method to analyse pharmaceutical compounds in solid matrices. Pharmaceuticals analysis has been carried out on a LC/MS-MS Quantum (Thermo Electron).

The main difficulty in this project is the lack of sample certified in researched analytes. Even with spiked solid matrices, it is still delicate to verify correctly the impact of extraction step because it does not reproduce a real sample.

What we propose here is a complete analytical method tested on sludge, soils and sediments.

2. APPARATUS, CHROMATOGRAPHIC AND DETECTION PARAMETERS

As we said in the previous report, the analysis of the method was carried out on an ion trap mass spectrometer LCQ (Thermo Electron) until last march. In march the transfer of the method on a LC/MS-MS Quantum (Thermo Electron) was carried out.

2.1 Chromatographic separation

The new HPLC apparatus consists of a Surveyor System, Autosampler, LC pump and a column oven (Thermo Electron, Courtaboeuf, France). The separation is performed on a Hypersil BDS C_{18} (125*2.1mm, 5µm) with a guard column BDS Hypersil C_{18} (10*2.1 mm, 5 µm) at flow-rate of 0.2 mL/min and 25°C thermostat. The injection volume is 35 µL. The table 1 presents the LC gradient conditions. The eluant A is composed of water + 10mM ammonium formate + 3% formic acid and the eluant B is composed of methanol + 10mM ammonium formate + 5% formic acid.

Time	***% A***	***% B***
0	95	5
30	0	100
32	0	100
37	95	5
40	95	5

Table 1 : the LC gradient conditions

2.2 Mass spectrometry detection

The detection is carried out using a Quantum (Thermo Electron, Courtaboeuf, France) tandem mass spectrometer. The mass spectrometer is operated in positive mode electrospray ionization (ESI+). The Selected Reaction Monitoring (SRM) mode is chosen for quantification. The spray voltage is fixed at 4000 V and the temperature of the capillary was set at 200°C.
For MS-MS analysis the pressure in the collision cell is set at 1.5 mTorr.
The fragmentation parameters (the SRM monitor ions and collision energies optimized) for each compound defined during the analytical development are the following :
The most intensive product ion from each precursor ion is selected and chosen as transition ion for detection and quantification.

	Compounds	Collision Energy (en V)	Ion parent m/z	Daughter Ion m/z
Segment 1	Paracetamol d_4	22	156.0	114.1
	Paracetamol	20	152.0	109.9
Segment 2	Phenazone	44	189.0	77.1
	Phenazone d_3	44	192.0	77.1
	Metoprolol	24	268.1	116.1
	Primidone	16	219.0	162.0
Segment 3	Propranolol	22	260.0	183.0
	Propranolol d_7	22	267.1	189.1
	Carbamazepine	26	236.9	193.9
	Ketoprofene	18	255.0	209.0
	Naproxène	18	231.0	185.1
	Bezafibrate	34	362.0	138.8
Segment 4	Diclofenac	38	295.9	214.0
	Diclofenac d_4	36	299.9	217.9
	Ibuprofene	20	224.1	161.0
	Gemfibrozil	10	268.1	233.1
	Gemfibrozil d_6	10	274.1	239.1

Table 2 : detection parameters for pharmaceutical compounds

3. CALIBRATION AND LIMITS OF QUANTIFICATION

3.1 Assessment

In the previous report results about linearity were presented. However for some compounds (phenazone and metoprolol) the results obtained were not satisfying because the response of these compounds in term of sensibility and repeatability was not correct.

Concerning the limits of quantification no results were presented in the previous report.

3.2 Working solutions preparation

All the working solutions are freshly prepared by dilution of stocked solutions.

As we said in the previous report, a working solution at 1 mg/L is prepared by dilution of stocked solution in methanol.
A mix of the internal standards is prepared by dilution of stocked solution in water/methanol (95/5). The concentration of this solution is 100 ng/mL.

The concentrations of the range changed since the previous report sent in January 2006. Indeed the aim of the transfer of the method on the LC/MS-MS Quantum was to obtain better results in term of sensibility and repeatability and actually better results were obtained. So before the transfer the concentrations of the range were between 50 and 750 ng/mL and now the concentrations of the range are between 5 (or 10 for some compounds) and 500 ng/mL.

Calibration standards are prepared with appropriate amounts of the working solution to achieve concentrations between 5 and 500 ng/mL.

	5	10	25	50	100	250	500
V of solution of mix pharmaceuticals(µL)	5	10	25	50	100	250	500

Table 3 : preparation of the range

All the standards are reduced to dryness under nitrogen. Then 1000 µL of the deuterium-labelled pharmaceutical compounds at 100 ng/mL. Standards are gently mixed prior to LC/MS-MS analysis.

3.3 Choice of the internal standard

As we have said before, 5 internal standards are chosen. For each pharmaceutical compound we need an internal standard in order to correct losses which can occur during the analysis.
So we need to determine which internal standard is associated.
So the internal standard paracetamol-d_4 is used to correct the following compounds :
- paracetamol
- ibuprofen

The internal standard phenazone-d_3 is used to correct the following compounds :
- phenazone
- metoprolol
- primidone
- carbamazepine

The internal standard propranolol-d_4 is used to correct the following compounds :
- propranolol
- ketoprofen
- bezafibrate
- naproxen

The internal standard diclofenac-d_4 is used to correct the following compound :
- diclofenac

The internal standard gemfibrozil-d_6 is used to correct the following compound :
- gemfibrozil

Figure of annexe 1 shows a LC-MS/MS chromatogram of solvent-base standard at 100 ng/mL for pharmaceutical compounds.

3.4 Results

3.4.1 Standards calibrations curves and repeatabilities of slopes

The following table shows the relative standard deviation on slopes obtained with 5 different calibration curves.

	Range 1	Range 2	Range 3	Range 4	Range 5	**Mean (n=5)**	**Deviation**	**RSD %**
Para	0.017138	0.011539	0.016302	0.013937	0.017729	0.015329	0.0025633	**16.7**
Phena	0.010967	0.006794	0.013512	0.009239	0.011109	0.010324	0.0024912	**24.1**
Meto	0.005007	0.005324	0.005283	0.004601	0.004657	0.004975	0.0003387	**6.8**
Primi	0.001530	0.001157	0.000933	0.001500	0.001142	0.001252	0.0002560	**20.4**
Propra	0.013919	0.011074	0.014645	0.014032	0.014896	0.013713	0.0015312	**11.2**
Carba	0.144399	0.124227	0.143238	0.180239	0.185548	0.155530	0.0262987	**16.9**
Keto	0.01303	0.016819	0.016994	0.016581	0.017648	0.016215	0.0018231	**11.2**
Napro	0.006453	0.007789	0.006657	0.007556	0.007062	0.007103	0.000570	**8.0**
Beza	0.003082	0.002881	0.002586	0.003170	0.002520	0.002847	0.0002897	**10.2**
Diclo	0.010320	0.013354	0.010645	0.011935	0.010004	0.011252	0.0013856	**12.3**
Ibu	0.000427	0.000442	0.000405	0.000423	0.000526	0.000444	0.0000475	**10.7**
Gem	0.009186	0.009106	0.007888	0.008253	0.007620	0.008410	0.0007084	**8.4**

Table 4: Statistical tests on slopes of 12 pharmaceuticals standard calibration curves

The whole of these results confirms the good linearity on calibration curves for all the compounds. The relative standard deviations are under 25% which is quite acceptable.
It is important to note that linearity is good for the 2 compounds phenazone and metoprolol that was not true before the transfer on the Quantum LC-MS/MS.

The recalculated values are presented in annexe 2. Relative standard deviations are under 18 % which is quite acceptable.

3.4.2 Repeatability on Limit Of Quantification (LOQ)

Tables 5, 6 and 7 illustrate criteria used for acceptable limit of quantification validation.

Target value	**5**	**5**	**5**	**5**
n = 10	Para	Beza	Propra	Diclo
1	5.628	6.211	5.637	5.154
2	5.166	6.089	4.440	5.281
3	5.032	5.930	4.608	5.607
4	4.691	5.296	4.359	5.753
5	4.535	5.561	4.350	5.688
6	4.577	5.307	4.484	5.832
7	4.465	5.115	4.214	5.672
8	4.393	5.359	4.322	5.880
9	4.396	5.193	4.127	5.724
10	4.363	5.271	4.181	5.891
Mean	**4.725**	**5.543**	**4.472**	**5.648**
Deviation	**0.419**	**0.417**	**0.434**	**0.246**
RSD %	**8.9**	**7.5**	**9.7**	**4.4**

Table 5 : Statistical tests for LOQ in ng/mL

Target value	**5**	**5**	**5**	**5**
n = 10	Gem	Phena	Meto	Kéto
1	4.679	3.975	6.162	5.413
2	4.605	5.468	5.684	4.577
3	4.087	5.671	5.521	5.362
4	4.626	4.499	6.252	4.663
5	3.888	5.727	6.093	4.374
6	4.147	5.587	6.245	4.647
7	4.167	5.506	5.536	4.097
8	4.813	4.725	5.767	4.683
9	4.254	4.251	5.508	4.271
10	4.708	5.235	5.579	4.679
Mean	**4.398**	**5.064**	**5.854**	**4.676**
Deviation	**0.323**	**0.645**	**0.350**	**0.424**
RSD %	**7.3**	**12.7**	**6.0**	**9.1**

Table 6 : Statistical tests for LOQ in ng/mL

Target value	**5**	**5**	**10**	**10**
n = 10	Carba	Napro	Primi	Ibu
1	6.099	4.910	11.540	8.737
2	5.782	4.830	11.359	9.284
3	5.732	4.611	11.708	9.871
4	5.227	4.706	11.526	9.229
5	5.169	4.635	11.455	8.117
6	5.079	4.371	11.786	9.174
7	5.059	4.299	11.645	7.877
8	4.821	4.860	11.493	8.448
9	4.901	4.710	12.263	9.093
10	4.983	4.398	12.382	8.191
Mean	**5.285**	**4.633**	**11.716**	**8.802**
Deviation	**0.431**	**0.214**	**0.344**	**0.633**
RSD %	**8.2**	**4.6**	**2.9**	**7.2**

Table 7 : Statistical tests for LOQ in ng/mL

According to the RSD and the concentrations obtained during this test of repeatability, the limits of quantification for pharmaceuticals compounds are the following :

- para, phena, meto, propra, carba, beza, diclo, napro, gem, keto : 5 ng/mL
- primi and ibu : 10 ng/mL

4. PROTOCOL

4.1 Assessment

4.1.1 Choice of the technique extraction

As we saw in the previous report the extraction technique selected is ultra-sonication. The sample is extracted 3 times with 20 mL of solvent during 15 minutes.

4.1.2 Choice of solvent extraction

As we saw in the previous report the solvent used for the extraction is acetonitrile with 0.1 % of NH_3.

4.1.3 Choice of the clean-up

The purification is carried out with the combination of SAX and HLB cartridges.

4.2 Step of purification

4.2.1 Conditions

In a first time, the only step of purification was studied in order to see what the efficiency of this step is. So for each type of matrice (sludge, soil and sediment), 3 extracts are carried out without any spiking. Then the extracts are spiked just before the purification. The spiking is 200 ng for each pharmaceutical compound and 100 ng for each internal standard.

4.2.2 Results

This test was carried out with the 3 different matrices (sediments, sludge and soils) but also with solvent.
The results presented below are the mean of the 3 values of recovery obtained for each type of matrice. Indeed for each extract recovery between the value found by the method and the theoretical value of 200 ng (the level of spiking) is calculated.

	solvent		**sludge**		**sediment**		**soil**	
	Recovery (%)	**RSD (%)**	**Recovery (%)**	**RSD (%)**	**Recovery (%)**	**RSD (%)**	**Recovery (%)**	**RSD (%)**
Para	128.7	3.5	125.9	1.1	122.9	0.4	120.6	1.8
Phena	77.7	24.5	112.0	11.7	115.0	4.5	116.9	3.1
Meto	81.3	16.9	103.3	11.5	96.3	8.7	94.4	3.6
Primi	76.0	17.5	90.2	3.0	81.9	12.9	84.9	4.6
Propra	107.9	2.2	111.0	0.9	104.5	2.9	105.0	1.8
Carba	**131.9**	5.6	110.5	4.8	104.0	6.9	106.7	8.0
Keto	78.9	6.5	83.8	6.9	89.9	5.0	103.0	3.0
Napro	75.7	20.7	89.3	19.2	84.3	2.6	104.7	5.0
Beza	107.1	7.5	86.4	11.4	91.4	10.7	107.6	2.4
Diclo	86.7	0.8	85.3	1.3	92.9	0.7	92.4	0.6
Ibu	80.0	21.7	114.0	20.3	122.1	3.0	126.9	11.4
Gem	95.2	0.5	120.1	12.2	82.8	3.3	96.4	6.6

Table 8: recoveries and RSD in % for samples spiked at 100 ng/g

The recoveries are good for the all of the pharmaceutical compounds and for the 4 types of matrices that is very satisfactory. Only one value is a little high (131.9% for carba with solvent) but it is not an aberrant value. Moreover the relative standard deviations are under 25 % which is quite acceptable.

4.3 Step of extraction

4.3.1 Conditions

In a second time, the step of extraction (and so all the protocol) was studied in order to study the efficiency of the step of extraction. So for each type of matrice (sludge, soil and sediment), 3 extracts are spiked just before the extraction. The same test is also carried out with samples of solvent and sand in order to see if we have difference between synthetic solutions and real matrices.

The spiking is 200 ng for each pharmaceutical compound and 100 ng for each internal standard.

4.3.2 Results

The results presented below are the mean of the 3 values of recovery obtained for each type of matrice. Indeed for each extract recovery between the value found by the method and the theoretical value of 200 ng (the level of spiking) is calculated.

4.3.2.1. Results for solvent and sand

	Solvent		Sand	
	Recovery in %	**RSD in %**	**Recovery in %**	**RSD in %**
Para	104.4	1.4	103.4	1.6
Phena	95.2	10.6	76.2	2.5
Meto	99.1	12.1	98.6	11.5
Primi	84.1	8.0	84.9	14.2
Propra	97.6	2.6	97.3	3.2
Carba	75.7	5.7	79.2	14.0
Keto	104.1	24.8	82.6	12.4
Napro	93.9	23.0	78.5	13.6
Beza	85.1	24.6	66.9	14.5
Diclo	109.1	1.1	110.4	1.7
Ibu	99.9	0.8	102.4	15.9
Gem	109.4	1.3	106.8	2.6

Table 9 : recoveries and RSD in % for samples of solvent and sand spiked at 100 ng/g

The results obtained here by spiking samples of solvent and sand in the beginning of the protocol are very correct. The recoveries are above to 75% except for one value. Indeed the recovery for beza with sand is 67%. This value is a little low and we can say that beza is not extracted correctly with this protocol.
The values in blue in the table correspond to the compounds which have their own internal standard.

4.3.2.2. Results for sludge

	Sludge 1		Sludge 2		Sludge 3	
	Recovery in %	RSD in %	Recovery in %	RSD in %	Recovery in %	RSD in %
Para	97.4	3.7	102.2	1.7	91.8	2.1
Phena	92.6	0.1	interference	interference	76.7	24.4
Meto	109.6	20.4	293.5	28.7	97.9	14.5
Primi	96.6	19.2	238.4	7.5	55.6	13.1
Propra	110.8	0.8	123.7	1.3	124.3	3.7
Carba	72.8	18.5	136.9	4.1	34.9	10.5
Keto	79.8	21.1	92.7	10.2	68.9	14.6
Napro	63.3	19.3	69.1	11.1	58.6	20.8
Beza	55.9	25.0	62.9	17.8	52.2	17.1
Diclo	113.6	1.5	126.0	2.3	133.4	1.1
Ibu	109.1	19.7	122.4	3.7	79.7	11.6
Gem	184.9	10.6	18.7	11.3	54.1	11.7

Table 10 : recoveries and RSD in % for 4 samples of sludge spiked at 100 ng/g

(Interference : Interference during the calibration)
We notice that for the compounds which have their own internal standard the recoveries are correct (values in blue in the table) except for the gem. Indeed for some compounds (para for example) the recoveries are good for the 3 different sludge.
But the test of extraction is not very convincing for other compounds. Indeed some values are not correct because the recoveries are a little low. We can suppose that the recoveries would be better with their own internal standards.

4.3.2.3. Results for soils and sediments

	Sediment 1		Sediment 2		Soil 1		Soil 2	
	Recovery in %	RSD in %	Recovery in %	RSD in %	Recovery in %	RSD in %	Recovery in %	RSD in %
Para	89.8	1.0	80.6	5.9	96.4	3.6	93.4	2.1
Phena	75.5	9.9	85.0	4.2	84.2	7.5	80.5	2.2
Meto	106.0	13.2	128.2	3.1	88.3	0.6	87.2	4.0
Primi	104.1	2.8	104.1	3.1	96.8	2.6	98.8	3.7
Propra	112.5	1.4	103.9	3.6	111.7	2.3	108.4	1.1
Carba	67.6	8.3	60.4	6.7	75.4	0.7	75.2	3.6
Keto	60.3	2.4	41.9	8.6	78.1	5.4	21.2	0.8
Napro	54.6	2.2	40.6	14.2	64.6	7.0	18.7	2.3
Beza	42.1	3.1	22.2	19.9	50.8	5.7	11.2	2.8
Diclo	114.9	1.2	119.5	2.2	106.7	0.2	110.0	1.2
Ibu	93.1	1.1	87.5	24.9	101.2	24.1	49.4	16.7
Gem	80.8	0.3	75.6	8.1	84.2	2.7	76.0	0.5

Table 8: recoveries in % for samples of soils and sediments spiked at 100 ng/g

As for sludge we notice that for the compounds which have their own internal standard the recoveries are correct. For some compounds (para, phena or propra for example) the recoveries are good for the 4 different matrices.
But for other compounds the test of extraction for sediments and soils is not very convincing. Indeed some values are not correct because the recoveries are a little low.

5. EVALUATION OF THE METHOD AND CONCLUSION

The analytical method developed on pharmaceutical compounds in sludge, soils and sediments is an efficient technique but not for all the compounds.

Concerning the calibration the whole of the results confirms the good linearity on calibration curves for all the compounds. The relative standard deviations are under 25% which is quite acceptable.

Concerning the purification the recoveries are good for the all of the pharmaceutical compounds and for the 3 types of matrices that is very satisfactory.

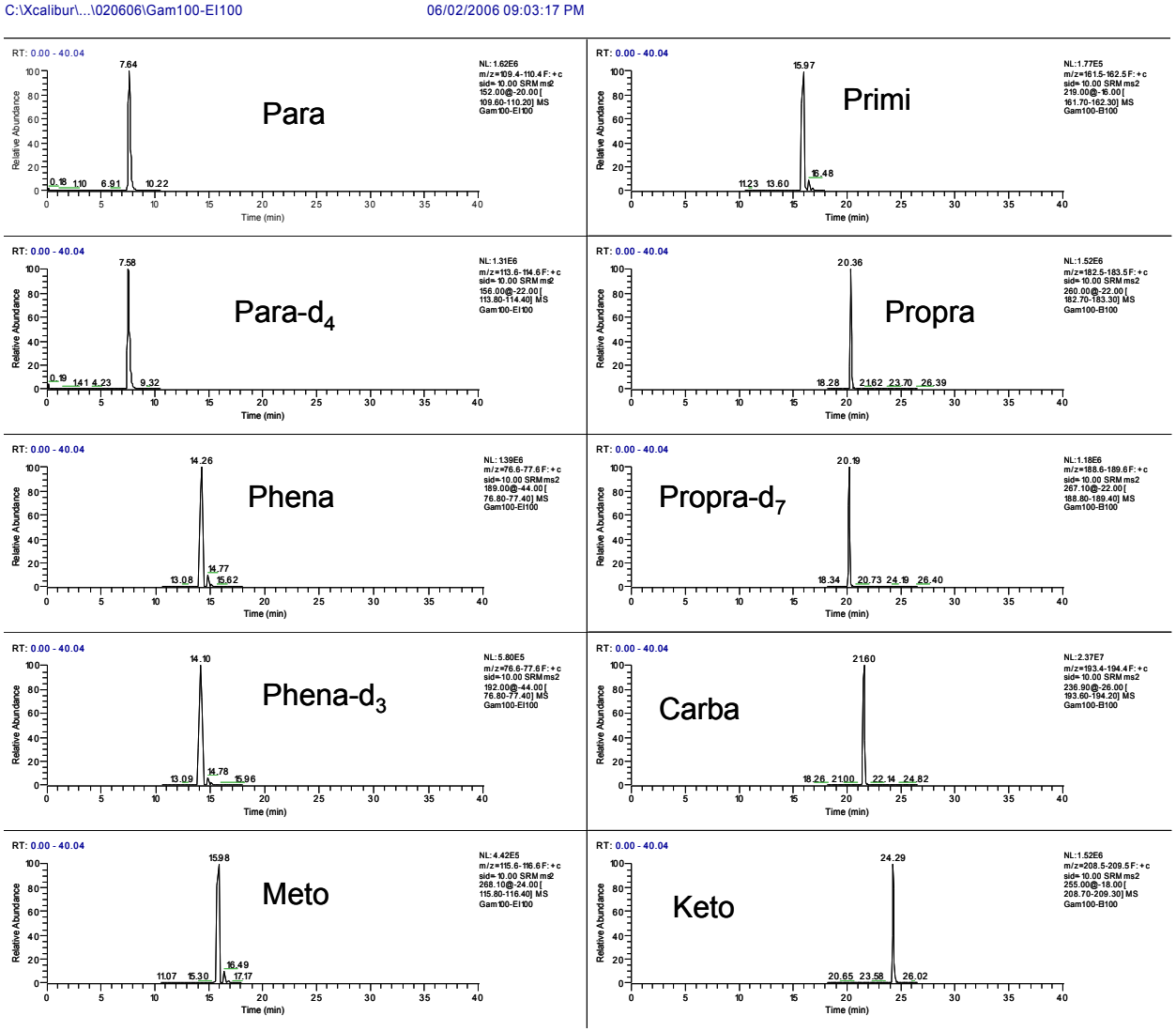
Concerning the extraction of solvent and sand the results obtained by spiking samples in the beginning of the protocol are very correct.

Concerning the extraction of real matrices the technique is efficient but not for all the compounds. Indeed for the 5 compounds which have their own internal standard the efficiency of the method is really convincing for the 3 different solid matrices.
But for the other compounds it is less convincing particularly for sludge. The recoveries are about around 50-60 % and we can suppose that these results would be better with appropriated internal standards.

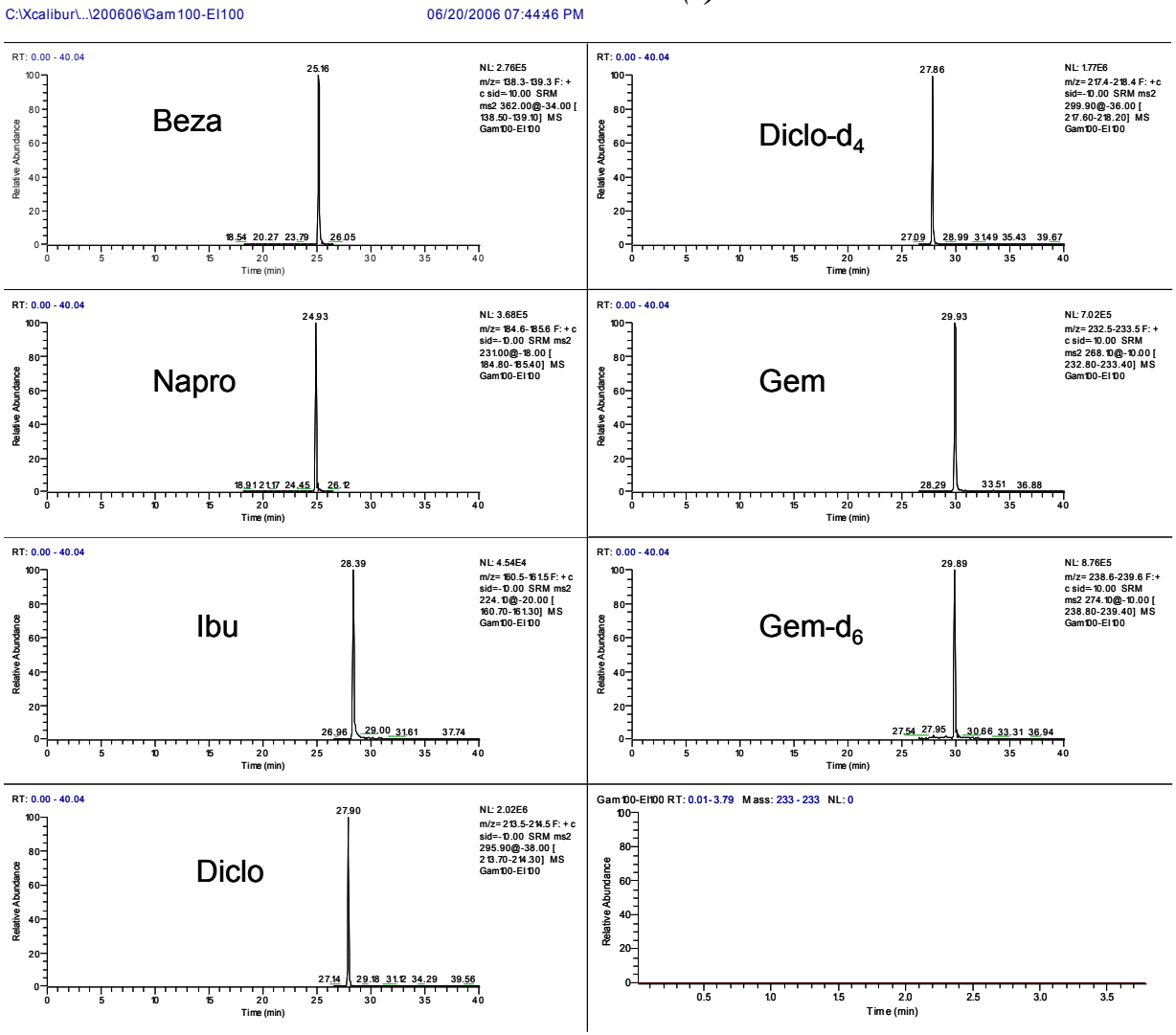
ANNEXES

Annexe 1 : LC-MS/MS chromatogram of solvent-base standard at 100 ng/mL

Pharmaceuticals (1)



Pharmaceuticals (2)



Annexe 2 : Pharmaceuticals calibration curves with recalculated values

Para

ng/mL	Gamme 1	Gamme 2	Gamme 3	Gamme 4	Gamme 5	Mean	Standard deviation	RSD	Deviation with the theorical concentration
5	4,721	4,533	4,665	5,082	5,409	**4,882**	**0,358**	**7,33%**	**2,36%**
10	10,103	10,103	10,256	8,162	10,425	**9,810**	**0,931**	**9,49%**	**1,90%**
25	24,946	25,383	25,340	26,432	23,549	**25,130**	**1,041**	**4,14%**	**0,52%**
50	51,684	50,386	52,445	56,134	47,330	**51,596**	**3,201**	**6,20%**	**3,19%**
100	100,476	105,477	95,593	100,252	95,239	**99,407**	**4,202**	**4,23%**	**0,59%**
250	256,562	258,540	259,781	256,651	259,370	**258,181**	**1,346**	**0,52%**	**3,27%**
500	491,508	485,578	491,920	487,370	498,678	**491,011**	**4,531**	**0,92%**	**1,80%**

Phena

ng/mL	Gamme 1	Gamme 2	Gamme 3	Gamme 4	Gamme 5	Mean	Standard deviation	RSD	Deviation with the theorical concentration
5	4,948	4,421	5,497	5,854	5,027	**5,149**	**0,548**	**10,65%**	**2,99%**
10	7,979	10,634	12,754	12,151	9,400	**10,584**	**1,959**	**18,51%**	**5,84%**
25	27,821	25,835	23,911	21,252	24,774	**24,719**	**2,426**	**9,81%**	**1,13%**
50	52,636	49,900	42,431	40,866	47,146	**46,596**	**4,947**	**10,62%**	**6,81%**
100	103,097	136,220	90,170	88,259	115,493	**99,255**	**12,674**	**12,77%**	**0,75%**
250	264,457	261,341	243,185	260,382	244,758	**254,825**	**10,037**	**3,94%**	**1,93%**
500	479,062	487,870	522,549	511,235	493,402	**498,824**	**17,726**	**3,55%**	**0,24%**

Meto

ng/mL	Gamme 1	Gamme 2	Gamme 3	Gamme 4	Gamme 5	Mean	Standard deviation	RSD	Deviation with the theorical concentration
5	5,696	5,764	4,382	4,168	5,190	**5,040**	**0,737**	**14,62%**	**0,80%**
10	9,074	9,445	8,817	9,777	7,226	**8,868**	**0,987**	**11,14%**	**11,32%**
25	28,152	27,210	23,875	28,688	24,969	**26,579**	**2,075**	**7,81%**	**6,32%**
50	44,754	52,698	60,279	50,318	57,542	**53,118**	**6,100**	**11,48%**	**6,24%**
100	86,296	86,627	113,167	104,186	114,953	**101,046**	**13,925**	**13,78%**	**1,05%**
250	268,660	264,387	245,326	242,639	240,006	**252,204**	**13,293**	**5,27%**	**0,88%**
500	497,369	494,633	484,154	535,754	490,113	**500,405**	**20,384**	**4,07%**	**0,08%**

Primi

ng/mL	Gamme 1	Gamme 2	Gamme 3	Gamme 4	Gamme 5	Mean	Standard deviation	RSD	Deviation with the theorical concentration
5	5,215	5,963	5,085	5,569	5,865	**5,539**	**0,387**	**6,98%**	**10,79%**
10	11,055	10,534	11,795	11,098	10,951	**11,087**	**0,455**	**4,10%**	**10,87%**
25	23,340	20,772	22,276	22,855	25,656	**22,980**	**1,780**	**7,75%**	**8,08%**
50	47,292	41,497	49,749	46,766	41,815	**45,424**	**3,621**	**7,97%**	**9,15%**
100	94,535	111,309	85,013	85,329	84,371	**92,111**	**11,519**	**12,51%**	**7,89%**
250	254,812	240,101	267,637	268,383	241,500	**254,487**	**13,618**	**5,35%**	**1,79%**
500	503,751	509,825	498,446	524,920	529,842	**513,357**	**13,533**	**2,64%**	**2,67%**

Propra

ng/mL	Gamme 1	Gamme 2	Gamme 3	Gamme 4	Gamme 5	Mean	Standard deviation	RSD	Deviation with the theorical concentration
5	3,847	3,834	3,940	4,835	4,837	**4,259**	**0,529**	**12,41%**	**14,83%**
10	9,580	9,861	9,770	10,137	9,733	**9,816**	**0,206**	**2,10%**	**1,84%**
25	26,697	28,144	25,989	24,593	25,596	**26,204**	**1,325**	**5,05%**	**4,82%**
50	54,474	58,149	56,145	50,860	49,613	**53,848**	**3,572**	**6,63%**	**7,70%**
100	109,855	107,271	111,893	97,850	103,298	**106,033**	**5,588**	**5,27%**	**6,03%**
250	272,831	259,600	244,567	268,258	258,171	**260,685**	**10,865**	**4,17%**	**4,27%**
500	462,717	473,641	487,696	483,468	488,752	**479,255**	**11,002**	**2,30%**	**4,15%**

Carba

ng/mL	Gamme 1	Gamme 2	Gamme 3	Gamme 4	Gamme 5	Mean	Standard deviation	RSD	Deviation with the theorical concentration
5	5,718	4,054	3,846	5,011	4,901	**4,706**	**0,762**	**16,18%**	**5,88%**
10	7,647	10,666	10,242	10,320	10,199	**9,815**	**1,226**	**12,49%**	**1,85%**
25	28,673	27,041	30,033	25,418	25,765	**27,386**	**1,955**	**7,14%**	**9,54%**
50	48,498	50,945	51,040	47,502	49,343	**49,466**	**1,539**	**3,11%**	**1,07%**
100	96,347	148,913	100,850	98,687	97,213	**98,274**	**1,970**	**2,00%**	**1,73%**
250	252,589	263,770	243,989	253,062	252,578	**253,198**	**7,024**	**2,77%**	**1,28%**
500	500,528	483,524	483,240	420,551	492,031	**489,831**	**8,216**	**1,68%**	**2,03%**

Keto

ng/mL	Gamme 1	Gamme 2	Gamme 3	Gamme 4	Gamme 5
5	5,231	5,322	5,263	6,183	6,114
10	10,403	10,556	9,910	10,739	11,115
25	23,783	28,294	24,386	22,455	21,799
50	47,447	47,131	49,942	40,536	45,282
100	94,655	76,024	98,025	99,622	84,046
250	274,791	250,035	248,485	231,757	252,169
500	483,688	522,639	503,990	528,709	519,475

Mean	Standard deviation	RSD	Deviation with the theorical concentration
5,623	**0,482**	**8,57%**	**12,45%**
10,545	**0,443**	**4,20%**	**5,45%**
24,143	**2,538**	**10,51%**	**3,43%**
46,068	**3,509**	**7,62%**	**7,86%**
90,474	**10,103**	**11,17%**	**9,53%**
251,447	**15,362**	**6,11%**	**0,58%**
511,700	**18,121**	**3,54%**	**2,34%**

Napro

ng/mL	Gamme 1	Gamme 2	Gamme 3	Gamme 4	Gamme 5
5	5,400	5,776	5,413	6,003	5,722
10	10,987	10,142	10,146	10,414	10,526
25	24,238	24,168	24,070	22,981	23,803
50	46,286	44,601	47,761	42,548	47,881
100	89,832	73,368	95,779	101,584	86,018
250	250,602	230,637	256,626	229,491	250,519
500	512,655	524,676	500,204	526,979	515,531

Mean	Standard deviation	RSD	Deviation with the theorical concentration
5,663	**0,257**	**4,53%**	**13,26%**
10,443	**0,347**	**3,33%**	**4,43%**
23,852	**0,514**	**2,16%**	**4,59%**
45,815	**2,260**	**4,93%**	**8,37%**
93,303	**6,827**	**7,32%**	**6,70%**
243,575	**12,586**	**5,17%**	**2,57%**
516,009	**10,686**	**2,07%**	**3,20%**

Beza

ng/mL	Gamme 1	Gamme 2	Gamme 3	Gamme 4	Gamme 5	Mean	Standard deviation	RSD	Deviation with the theorical concentration
5	6,121	4,717	5,668	5,509	5,774	**5,558**	**0,521**	**9,37%**	**11,16%**
10	9,762	10,758	10,092	10,647	10,068	**10,265**	**0,422**	**4,11%**	**2,65%**
25	23,848	27,181	24,079	22,728	23,669	**24,301**	**1,690**	**6,95%**	**2,80%**
50	45,826	51,138	44,959	48,256	46,162	**47,268**	**2,479**	**5,25%**	**5,46%**
100	92,381	84,714	93,271	91,370	94,734	**91,294**	**3,880**	**4,25%**	**8,71%**
250	240,583	250,332	269,148	261,244	251,001	**254,462**	**10,992**	**4,32%**	**1,78%**
500	521,478	511,159	492,783	500,246	508,593	**506,852**	**10,921**	**2,15%**	**1,37%**

Diclo

ng/mL	Gamme 1	Gamme 2	Gamme 3	Gamme 4	Gamme 5	Mean	Standard deviation	RSD	Deviation with the theorical concentration
5	5,807	5,365	5,137	5,895	4,938	**5,428**	**0,415**	**7,65%**	**8,57%**
10	9,829	10,010	9,885	9,713	10,317	**9,951**	**0,231**	**2,32%**	**0,49%**
25	23,369	24,448	25,354	24,879	25,680	**24,746**	**0,901**	**3,64%**	**1,02%**
50	49,068	48,571	48,681	47,603	48,870	**48,559**	**0,567**	**1,17%**	**2,88%**
100	92,961	97,473	98,605	85,385	93,927	**93,670**	**5,197**	**5,55%**	**6,33%**
250	246,007	246,769	252,793	257,860	262,149	**253,116**	**6,983**	**2,76%**	**1,25%**
500	512,959	507,362	499,545	508,666	494,119	**504,530**	**7,573**	**1,50%**	**0,91%**

Ibu

ng/mL	Gamme 1	Gamme 2	Gamme 3	Gamme 4	Gamme 5
5	5,641	5,670	8,437	5,783	6,074
10	10,490	9,275	11,558	11,016	10,431
25	22,825	26,877	24,832	21,968	23,699
50	45,216	48,305	46,965	45,322	43,583
100	94,454	86,888	90,759	92,161	86,864
250	269,024	251,237	241,145	253,735	257,543
500	492,348	511,748	519,741	510,013	511,807

Mean	Standard deviation	RSD	Deviation with the theorical concentration
5,792	**0,198**	**3,41%**	**15,84%**
10,554	**0,848**	**8,04%**	**5,54%**
24,040	**1,908**	**7,94%**	**3,84%**
45,878	**1,809**	**3,94%**	**8,24%**
90,225	**3,330**	**3,69%**	**9,77%**
254,537	**10,122**	**3,98%**	**1,81%**
509,131	**10,112**	**1,99%**	**1,83%**

Gem

ng/mL	Gamme 1	Gamme 2	Gamme 3	Gamme 4	Gamme 5
5	5,210	5,780	5,317	4,831	4,645
10	10,246	10,043	9,583	10,176	10,465
25	25,580	22,998	25,154	26,210	25,046
50	49,760	47,719	48,864	48,172	51,150
100	89,760	95,519	100,541	98,911	98,133
250	249,269	247,171	244,171	255,923	258,567
500	510,176	510,770	506,370	495,778	491,994

Mean	Standard deviation	RSD	Deviation with the theorical concentration
5,157	**0,443**	**8,59%**	**3,13%**
10,103	**0,328**	**3,25%**	**1,03%**
24,998	**1,208**	**4,83%**	**0,01%**
49,133	**1,365**	**2,78%**	**1,73%**
96,573	**4,218**	**4,37%**	**3,43%**
251,020	**6,037**	**2,41%**	**0,41%**
503,018	**8,610**	**1,71%**	**0,60%**