MACHEREY-NAGEL
Chromatography application note

MN application note 04/2018

Determination of pharmaceuticals from serum

MACHEREY-NAGEL application department · Dr. H. R. Wollseifen

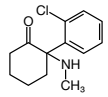
Abstract

This application note describes the determination of pharmaceuticals from serum using solid phase extraction for analyte enrichment and for sample clean-up. The eluates from SPE are finally analyzed by HPLC-MS/MS.

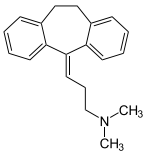
Introduction

Nowadays, people are suffering from various diseases. Therefore, they are prescribed many types of pharmaceuticals as part of their treatment, for instance anesthetics, antibiotics, anticholinergics, anticonvulsants, etc. In order for the treatment to be successful, it is necessary to keep controlling the levels of the pharmaceuticals to provide an accurate dosage. This leads to an increasing demand for the development of accurate and sensitive analytical methods for the pharmaceuticals from serum to protect human health.

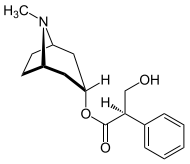
In this application note a SPE method using CHROMABOND® HLB for the determination of pharmaceuticals from serum was developed. The identification and quantification of the analyzed pharmaceuticals were finally carried out by ESI mass spectrometry on a NUCLEOSHELL® PFP.

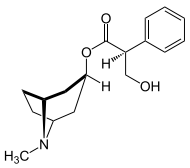


Anesthetic (Ketamine)

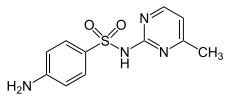


Antidepressant (Amitriptyline)

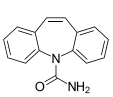




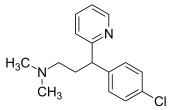
Anticholinergic (Atropine)



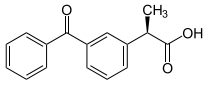
Antibiotics (Sulfamerazine)



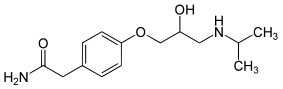
Anticonvulsant (Carbamazepine)



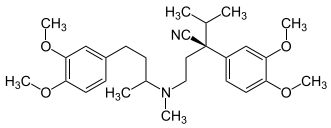
Antihistamine (Chlorpheniramine)



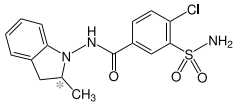
Anti-inflammatory drugs (Ketoprofen)



Beta blocker (Atenolol)



Calcium channel blockers (Verapamil)



Diuretic (Indapamide)

Figure 1: Compounds of interest.

Solid phase extraction

Column:

CHROMABOND® HLB, 1 mL, 30 mg, (REF 730921)

Conditioning:

1 mL methanol, 1 mL water

Sample application:

1 mL spiked serum sample is passed through the column by vacuum

Washing:

1 mL water

Drying:

10 min with vacuum

Elution:

2 mL methanol

Eluent exchange:

Evaporate eluate to dryness at 40 °C under a stream of nitrogen and reconstitute in 1 mL water – acetonitrile (95:5, v/v)

Subsequent analysis: HPLC-MS / MS

Chromatographic conditions

Column:

EC 50/2 NUCLEOSHELL® PFP, 2.7 µm, (REF 763532.20)

Eluent A:

0.1 % formic acid in water

Eluent B:

0.1 % formic acid in acetonitrile

Gradient:

5–95 % B in 7.5 min, 95 % B for 1 min, 95–5 % B in 0.5 min, 5 % B for 5 min

Flow rate:

0.3 mL/min

Temperature:

30 °C

Injection volume:

5 µL

MACHEREY-NAGEL GmbH & Co. KG · Valencienner Str. 11 · 52355 Düren · Germany
www.mn-net.com · info@mn-net.com

Determination of pharmaceuticals from serum

MS conditions

API 5500 (AB Sciex), ion source ESI, positive ionization mode, scan type SRM, detection window 90 s, curtain gas 40 psig, ion spray voltage 5500 V, temperature 500 °C, nebulizer gas 45 psig, turbo gas 45 psig, CAD medium

SRM transitions

Analyt	Retention time [min]	$[M-H]^-$	Q_1 (Quantifier)	Q_2 (Qualifier)
Atenolol	1.12	267.2	145.2	74.1
Sulfapyridine	1.72	242.9	130.9	96.9
Atropine	1.81	290.2	124.2	93.0
Sulfamerazine	1.82	265.1	156.0	91.9
Ketamine	1.87	238.2	125.1	179.1
Chlorpheniramine	2.27	275.1	230.0	167.0
Sulfachloropyridazine	2.47	285.1	156.0	91.9
Sulfadoxine	2.63	311.1	156.0	92.1
Sulfamethoxazole	2.70	254.1	155.8	91.8
Propanolol	2.74	260.2	116.2	182.9
Diphenhydramine	2.94	256.1	166.9	152.1
Amitriptyline	3.04	278.2	223.0	91.0
Sulfaquinozaline	3.14	301.1	156.1	92.1
Nortriptyline	3.32	264.2	232.9	91.1
Verapamil	3.36	455.2	165.0	150.1
Trimipramine	3.41	295.2	100.1	58.0
Carbamazepine	3.50	237.1	194.1	193.0
Clomipramine	3.67	315.1	86.1	58.0
Indapamide	3.77	366.1	132.1	91.1
Ketoprofen	4.28	255.1	77.0	105.0

Table 1: SRM transitions for pharmaceuticals.

Chromatogram

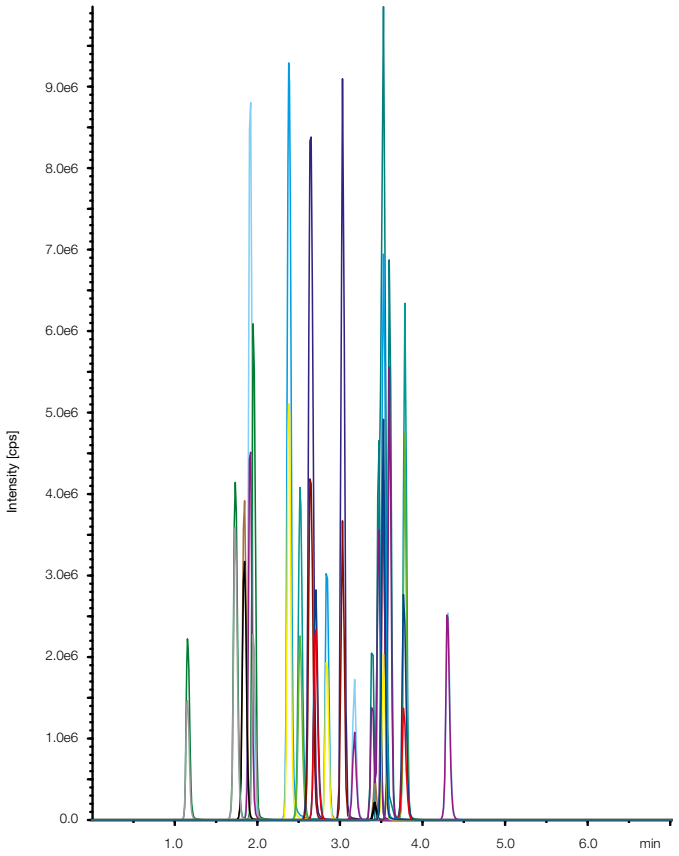


Figure 2: Chromatogram of serum sample spiked with 10 ng/mL for each pharmaceutical.

Recovery rates

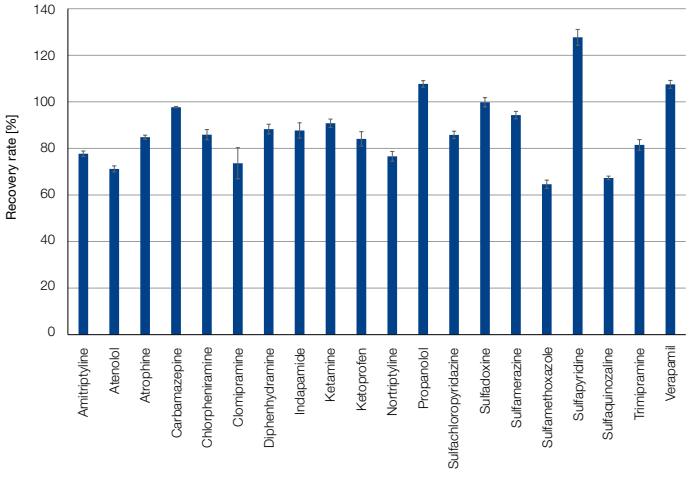


Figure 3: Recovery rates for solid phase extraction method of pharmaceuticals from serum.

Determination of pharmaceuticals from serum

Conclusion

The results show that the determination of pharmaceuticals from serum could be carried out successfully with all the tested products as presented in figure 3. By using SPE with CHROMABOND® HLB it was possible to recover more than 85 % of pharmaceuticals from serum on average with good reproducibility. Regarding the different types of pharmaceuticals the average recovery rates were: for anesthetics 90.8 %, for antibiotics 94.4 %, for anticholinergics 84.8 %, for anticonvulsants 97.7 %, for antidepressants 77.4 %, for antihistamines 87.1 %, for anti-inflammatory drugs 84.1 %, for beta blockers 89.5 %, for calcium channel blockers 107.5 % and for diuretics 87.7 %. The identification and quantification of pharmaceuticals in the solid phase extracts were carried out by ESI mass spectrometry on an EC 50/2 NUCLEOSHELL® PFP column. The chromatogram in figure 2 shows the results of solid phase eluate spiked with 10 ng/mL serum for each pharmaceutical.

In summary, the presented application describes a quick and convenient method for the determination of pharmaceuticals from serum with a SPE procedure.

Additional information

The following applications regarding “Determination of pharmaceuticals from serum” and further applications can be found on our online application database at *www.mn-net.com/apps*

SPE: MN Appl. No. 306510

HPLC: MN Appl. No. 128200

Product information

The following MACHEREY-NAGEL products have been used in this application note:

REF 763532.20, EC 50/2 NUCLEOSHELL® PFP, 2.7 µm,

REF 730921, CHROMABOND® HLB, 1 mL, 30 mg

REF 702293, Screw neck vials N 9, 1.5 mL

REF 702107, N 9 PP Screw cap, yellow, center hole, silicone white / PTFE red

Image credits