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A NEW EXTRACTION AND DETECTION METHOD FOR ANTI-DIABETIC COMPOUNDS AND THEIR METABOLITES FROM WWTP SLUDGE SAMPLES

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Introduction

The occurrence of pharmaceutical compounds in wastewater and surface water has been studied in recent years, thus indicating the inefficiency of conventional treatment plants to block the penetration of these emerging contaminants into the environment. The lack of adequate analytical methodologies and the presence of fractions of these micropollutants adsorbed in the sewage sludge, make it difficult to understand the distribution and evaluate their behavior, especially of biodegradable compounds during the wastewater treatment process. The sludge resulting from domestic and industrial wastewater treatment may contain organic residues with undesirable consequences, due to biological and chemical contaminants adsorbed, but under certain conditions can also be used as a natural fertilizer / fertilizer. Before use, the sludge must be evaluated for organic residues (PAH, PCB, AOX), pharmaceuticals, heavy metals, in addition to other potential pathogenic factors (microbes).

Materials and methods

The parameters obtained during the analytical chromatographic separation process included: Eclipse C18 chromatographic column (100x2mm, 3.4 µm), column temperature 30 ° C, injected extract volume 10 µL, mobile phase composition 0.1% formic acid: acetonitrile with gradient elution, test running time 9 min. For the detection of analytes with the quadruple triple mass spectrometer (QQQ MS) detector, the following optimal operating parameters were established: positive ionization mode (ESI +), nitrogen temperature at source 300^0C, type of ESI source (electrospray), nitrogen flow for drying ions 10 L/min, nebulizing gas pressure 50 psi, voltage applied to the capillary 3000 V, collision energies 10-25 V, fragmentation voltages 80-120 V. Also, a voltage of 4 V was applied on the collision cell and for each compound, 2 MRM transitions (multiple reaction monitoring) were recorded from the precursor ion to the most abundant product ions (Quantifier, qualifier). For the processing of wet sludge samples, the following optimal parameters of solid - liquid selective extraction were established: lyophilization time 24h, sample mass 0.5g, ultrasonic extraction time, 15 minutes, methanol / acetone

extraction solvent (1: 1), solvent volume extraction speed 10 mL, speed and spin time (3000 rpm, 10 min) (Figure 1).

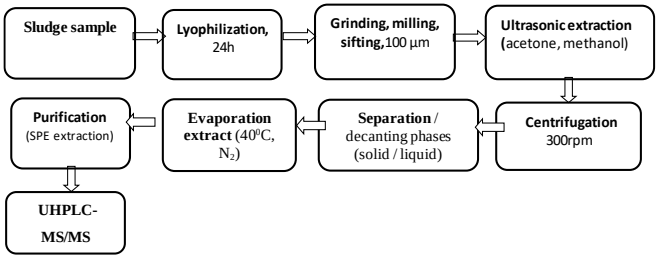

Fig. 1. A. Diagram for antidiabetics extraction from sludge by ultrasonication, centrifugation and SPE purification

Results and conclusions

For the extraction and analysis of antidiabetics from sewage sludges a new method was developed, optimized and validated. The linear regressions were obtained using the method of the external standard and the afferent coefficients of determination (R2) were higher than 0.99 on the calibration range 1-100 ng/mL, respectively, 2-200 ng/g in the sludge samples. The limits of quantification, calculated by ratio of signal to noise which, varied in the range 0.1-1.8 ng/g. These limits allow the quantification of antidiabetics in sludge samples taken from treatment plants, at the level of traces (ng/g). RSD values obtained for repeatability ranged from 2.6% to 8.1% for all compounds. For intermediate precision experiments, the RSD was below 12.0%. The results obtained from repeatability and intermediate precision experiments indicate that the method was repeatable and reproducible with standard deviations <15%. By testing the method on the real sewage sludge samples with known addition of the standard, recovery yields were calculated in accordance with the chromatographic methods, with values in the range of 74.6 to 88.9%. The obtained method presents the accuracy corresponding to the LC methods.

Table 1. Calibration data for sewage sludge antidiabetics

Compound	R^2	Calibration range (ng/mL)	Recovery (%)	Precision (%)		LOQ, ng/g
				RSD_r	RSD_R	
Metformin	0.9941	1-100	74.6	8.15	12.03	0.2
Glipizid	0.9974	1-100	84.6	4.66	10.30	0.1
Gliclazid	0.9969	1-100	87.5	3.25	8.64	0.1
Gliburid	0.9977	1-100	79.6	5.04	11.10	0.2
Glimperid	0.9982	1-100	88.9	2.57	7.77	0.1
Guaniluree	0.9982	5-100	77.6	4.79	10.54	1.8

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