QUECHERS METHOD FOR SIMULTANEOUS DETERMINATION OF VETERINARY DRUGS AND PESTICIDES ANAYLSİS İN MİLK BY LC-MS/MS

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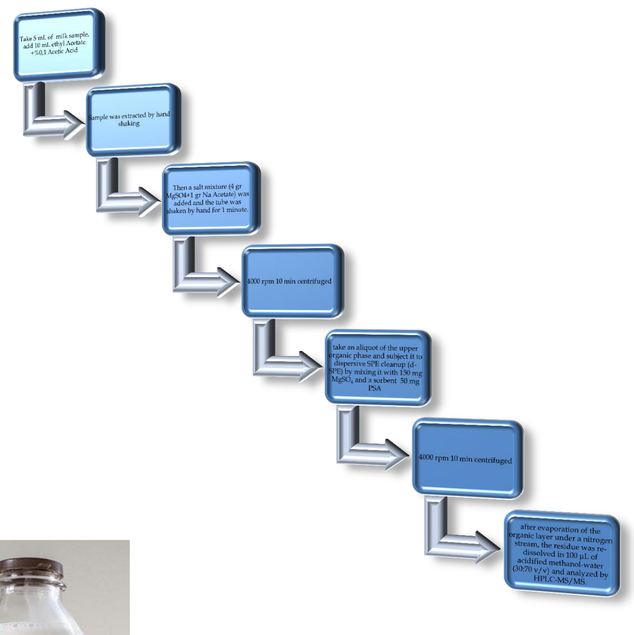
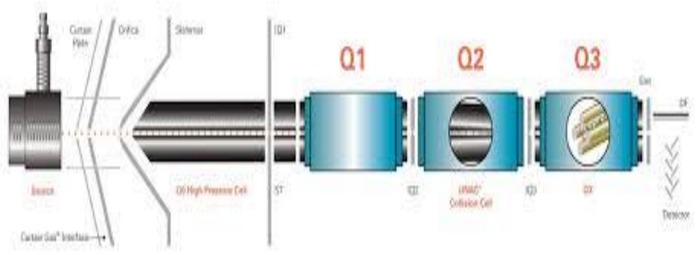
Introduction

Veterinary drugs are used for disease treatment and prevention and also for improvement of growth and feeding efficiency. Incorrect use of these drugs may cause accumulation of such drugs in the animal body and intake by human. Therefore they should be checked to protect the agricultural environment and food industry Since there are many veterinary drugs to be monitored, development of methods enabling a wide number of drugs with multiple classes by a single analysis procedure is quite important. In this study, we prepared milk samples by using a procedure based on a simple liquid–liquid extraction. This method utilized a simple and quick sample preparation procedure using a single extraction step. (A. Kaufmann 2014 and R. Souza 2016) Additionally, reduced amount of chemicals and steps in sample preparation phase together with the avoidance of a sample clean-up step simplify the sample pretreatment and reduce the total laboratory costs. The proposed method reduced costs of analysis and improved the simplicity and provided high recoveries of compounds of various polarities. Using the method, milk samples were analysed for both veterinary drugs and pesticide residues simultaneously (Y. Zhang 2015 and H. G. Mol 2008) using liquid chromatography tandem mass spectrometry (LC–MS/MS).

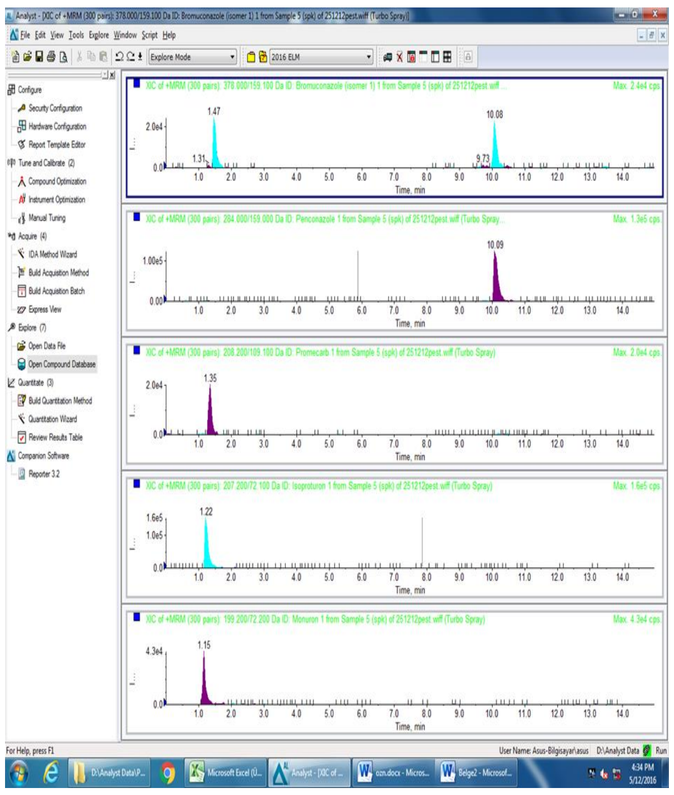


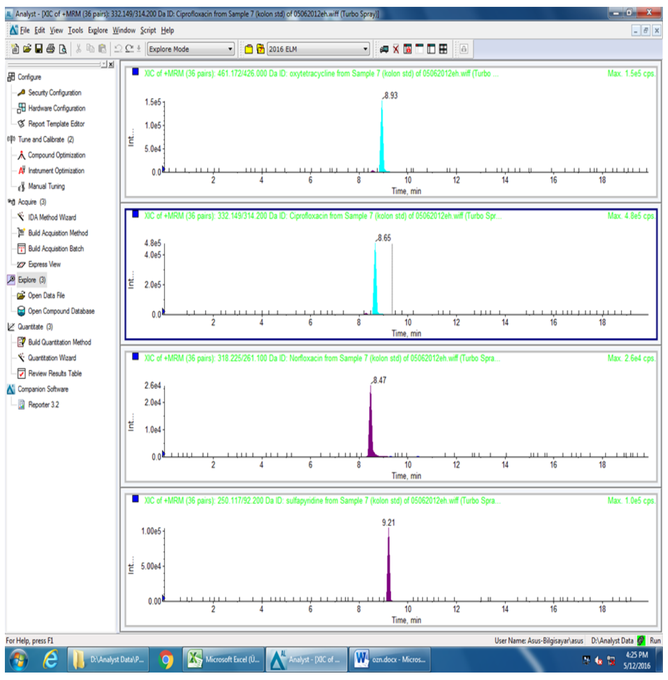


Kolon	**Hypersil Gold C18 100x2,1mm 1,9 µm**			
Enjeksiyon hacmi	20 mcL			
Gradient program	time	% A Mobil faz (su)	%B Mobil faz (metanol)	Flow(mL/min)
	0	50	50	0,3
	0.5	50	50	
	3.5	0	65	
	5.5	0	100	
	7	0	100	
	7.01	50	50	
	10	50	50	
Kolon sicakligi	30 °C			
Ionization Mode	ESI positive			
Spray Voltage	4 kV			
Capillary Temp	300°C			
Sheath Gas	35 au (arbitary units)			
Auxiliary gas	10 au			
Skimmer Voltage	18 V			
Capillary Voltage	35 V			
Tube lens voltage	95 V			
Heater Temp	305°C			
Capillary temp	300°C			
Automatic gain control (AGC)	$1x10^6$ ions			
Mass resolving power	25,000 FWHM			
Scan Time	0,25 s full MS			
Mass resolving power	10,000 FWHM			
Scan rate	0,56 Hz			
Full scan *m/z*	100-1000			
Instrument control and data processing	XCalibur 2.2.1 softwarw (Theormo Fisher Scientific, Les Ulis, France) with Qual and Quanbrowser. Genesis peak dedection was applied ToxID™ 2.1.1 was using for screening and LCQuan™2.6 (Theormo Scientific)			

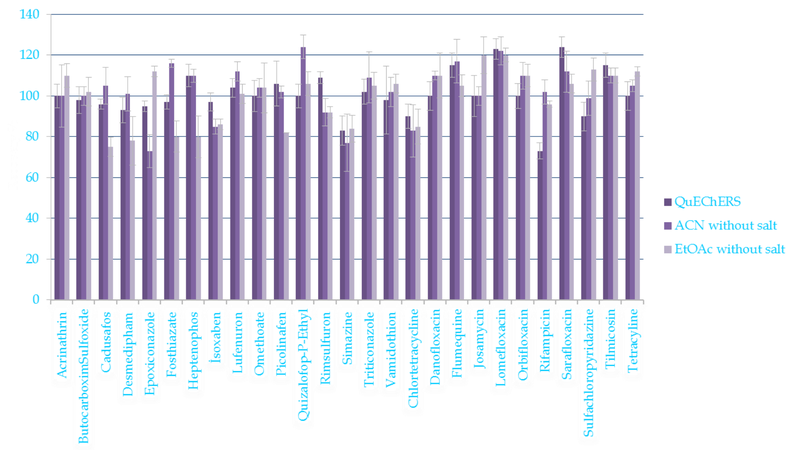
VALİDATHİON STUDY

Compounds	r^2	LOQ	%Recovery	% RSD	%RSDr	Relative	Matrix
		(µg/kg)	50 (µg/kg)	50 (µg/kg)		Uncertainty %	Effect
2,4 D (Negatif)	0.997	11	108	5	13	32	0,81
2 4 5 T	0.998	9	93	9	11	23	0,92
2,4 Dimethylanilin	0.998	8	97	8	11	35s	0,99
Acetamiprid	0.997	10	96	7	11	33	0,92
Acrinathrin	0.998	11	104	7	11	35	0,81
Alachlor	0.997	11	92	3	13	28	0,81
Amitraz	0.997	9	76	3	14	28	0,81
Atrazine	0.997	11	91	3	9	30	0,85
Azoxystrobin	0.998	11	110	4	14	30	0,92
Buprofezin	0.997	9	88	8	10	26	0,81
ButocarboximSulfoxide	0.997	11	102	7	7	33	1,06
Cadusafos	0.997	11	95	5	6	30	0,85
Carbaryl	0.997	11	89	4	9	28	0,85
Carbendazim	0.997	12	113	4	11	30	1.10.
Desmedipham	0.998	10	87	7	14	0	0,88
Di-Allate	0.998	10	110	6	14	26	1,08
Ethofumesate	0.998	11	100	7	13	28	0,86
Etoxazole	0.998	11	101	4	11	28	0,85
Ethoxyquin	0.998	10	98	4	11	30	0,86
Famoxadone	0.998	11	117	5	12	32	1,03
Fenamidone	0.998	11	91	6	10	26	0,82
İmidacloprid	0.999	9	109	6	15	26	0,92
İndoxacarb	0.999	10	93	8	16	30	0,9
İoxynil	0.999	10	81	5	8	30	0,81
İprovalicarb	0.999	10	104	7	15	32	0,99
İsazofos	0.999	12	89	11	13	26	0,85
İsoproturon	0.999	12	97	6	11	38	0,81
İsoxaben	0.997	11	96	5	8	30	0,99
Lufenuron	0.997	10	94	4	13	30	0,88
Malathion	0.999	12	89	6	13	33	0,94
Simazine	0.997	11	99	3	11	23	0,86
Spiroxamine	0.999	8	82	2	14	32	0,82
Sulfosulfuron	0.997	8	111	6	19	30	0,99
Tebuconazole	0.999	10	99	5	9	25	0,85
Tepp	0.997	9	105	6	11	29	0,96
Terbutryn	0.997	12	102	3	11	28	0,86
Terbuthylazine	0.997	10	116	4	6	26	1,06
Tetrachlorvinphos	0.997	11	90	4	14	28	0,9
Thiabendazole	0.997	12	96	5	13	30	0,88
Zoxamide	0.997	12	85	3	15	25	0,82
Ciprofloxacin	0.997	7	97	13	9	38	0,81
Clindamycin	0.997	11	109	10	12	34	0,96
Chlortetracycline	0.999	10	99	6	9	14	0,84
Danofloxacin	0.999	10	88	8	9	24	0,82
Difloxacin	0.999	9	96	6	10	15	0,9
Doxycycline Hydrate	0.999	10	96	8	10	23	0,84
Flumequine	0.998	9	93	10	11	22	0,95
Josamycin	0.998	9	97	9	12	22	0,94
Lomefloxacin	0.999	10	99	8	10	22	0,8
Orbifloxacin	0.999	9	94	7	11	20	0,96
Oxolinic Acid	0.999	11	102	6	15	20	0,92
Oxytetracycline	0.999	11	95	6	10	23	0,94
Rifampicin	0.997	10	98	5	9	15	0,81
Sarafloxacin	0.997	10	102	5	11	16	0,87
Sulfachloropyridazine	0.997	9	80	9	10	24	0,96
Sulfaquinoxaline	0.998	10	78	9	9	24	0,89
Sulfadiazine	0.997	8	98	5	10	18	0,88
Sulfamerazine	0.999	8	75	7	9	19	0,94
Tilmicosin	0.999	10	105	4	10	18	0,94
Tetracyline	0.999	10	96	5	9	23	0,82





Recovery data of different solvent extraction procedures.



Results and Discussion

Validation Study

The selectivity of the method was assessed by duplicate analysis of seven blank milk samples. No peaks of interfering compounds were observed within the intervals of the retention time of the analytes in any of these samples. Linearity was evaluated from the calibration curves by triplicate analyses of blank milk samples fortified with the analytes at five (0.0, 0.01-0.025-0.05-01-0.2 mg/kg) concentration levels. Linearity was expressed as the coefficient of linear correlation (r) and from the slope of the calibration curve. The linearity of the analytical response across the studied range was excellent, with correlation coefficients higher than 0.997 for all analytes.

Conclusions

A multi-class multi-residue procedure with LC-MS/MS detection has been developed and validated to determine and quantify veterinary and pesticide residues in milk. A simple sample preparation method was used involved liquid extraction salting out procedures in ethyl acetate system, without clean-up steps, and shortening the sample preparation time. Validation of the method was performed according to Commission Decision 2002/657/EC. The method was characterized by good results in terms of recovery, reproducibility and repeatability allowing the detection of veterinary drug and pesticide residues below the recommended analytical level. Based on these results, LC-MS/MS method with ethyl acetate extraction showed the suitability for sensitive quantification of veterinary and pesticide residues in milk samples for food safety applications. The validated method was applied on 220 real commercial samples.

References

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