Extractables Detection in Rubber Plug Products

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Introduction

For E&L researchers, injections and injectable suspensions are high & medium risk products, for which the most complex components are thermoplastic elastomers. A rubber plug is commonly used in cillin bottles, manufactured with polyisoprene rubber, butyl rubber, halogenated butyl rubber and many otherrubber-like materials.

There is no doubt that HRMS is more commonly used for E&L compound screening and identification, partially simplified with AET value (Analytical Evaluation Threshold), which is based on the SCT and is the threshold *at-or-above* which a chemist should begin to identify a particular leachable or extractable for potential toxicological assessment.

Considering the regulatory detection requirements and applicable coverage, an LC/TQ system is the gold standard for targeted compound detection and quantitation where a MRM method can be used for E&L research.

In this study, we investigated the detection of 35 compounds in rubber plugs using an Agilent 6470 triple quadrupole LC/MS system (LC/TQ). These compounds include antioxidants, slip agents, and vulkacits, which are the most conventional and widely used additives in the manufacture for elastomers.

This method aims to test the feasibility of LC/TQ technology for the measurement of E&L, to help manufacturers to evaluate their elastomer products and set up quality control standards – at relatively lower cost than HRMS platforms.

The 6470 triple quadrupole LC/MS coupled to the 1290 Infinity II HPLC

Experimental

Sample preparation

For 1g of rubber stopper sample,

1. Cut into pieces with a diameter of about 5mm
2. Microwave extract with 10ml of dichloromethane at 40 ° C for 45min,
3. Dry with nitrogen then dissolve with 1ml of isopropanol
4. Solvent extracts are injected directly into the LC-TQ system.

Agilent 1290 Infinity II UHPLC System	
Column	Agilent ZORBAX RRHD Eclipse Plus C8, 3.0* 150 mm, 1.8 µm
Column temperature	45 °C
Injection volume	2 µL
Autosampler temp	4 °C
Mobile phase	A) Water(4.5mM NH_4Formate + 0.5mM NH_4F + 0.1% formic acid) B) 80%Methanol + 20% isopropanol (4.5mM NH_4Formate + 0.5mM NH_4F + 0.1% formic acid)
Flow rate	0.4 mL/min
Stop time	25min

Agilent 6470 LC/TQ System	
Drying gas temperature	325 °C
Drying gas flow	10 L/min
Sheath gas temperature	350 °C
Sheath gas flow	11 L/min
Nebulizer pressure	45 psi
Capillary voltage	4000 V(pos)/3500V(neg)
Nozzle voltage	0 V(+)/500 V(-)
Delta EMV	200 V
Polarity:	Positive/Negative

Experimental

Trap column configuration

E&L compounds, especially antioxidants, were found as contamination at very low concentrations in mobile phase solvents, which may give false positive results if not taken into account. So, the use of a C18 trap column (Agilent ZORBAX Eclipse Plus C18, 2.1* 50 mm, 1.8 µm) should be situated between the Binary Pump and the Autosampler. The addition of a trap column is used to delay interferences from the mobile phase, which will be eluted about 0.5min later than target compounds of interest.

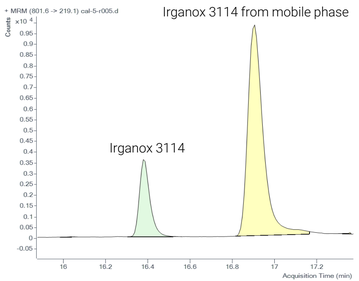
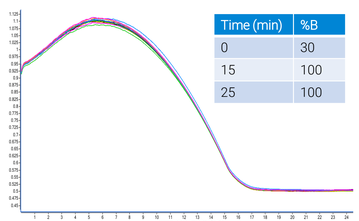


Fig 1. Delayed peak by trap column



Time (min)	%B
0	30
15	100
25	100

Fig 2. Reproducibility of binary pump pressure profiles

Table 1. MRM list for 35 E&L compounds

Compound Name	Precursor Ion	Product Ion	Polarity
2,4-Di-tert-butylphenol	205.1	189.1	Negative
	205.1	173.1	
9-Octadecenamide	282.3	265.3	Positive
	282.3	247.3	
Benzothiazole	136	109	Positive
	136	65	
BHT	219.1	219	Negative
	219.1	203.1	
BHT-CHO	235.2	179	Positive
	235.2	57.1	
BHT-COOH	251.2	195	Positive
	251.2	57.1	
BHT-OH	235	217.2	Negative
	235	160.1	
Bis(diisobutyltiocarbamoyl) disulfide	409.2	172.1	Positive
	409.2	116	
BPA	227	212.1	Negative
	227	133.1	
Cyanox 2246	358.3	229	Positive
	358.3	121	
Cyanox 425	386.3	257.1	Positive
	386.3	191.1	
Dipentamethylenethiuram disulfide	321	160	Positive
	321	128	
Dipentamethylenethiuram tetrasulfide	385	204	Positive
	385	172	
Disulfiram	297.1	116	Positive
	297.1	88	
Erucamide	338.3	321	Positive
	338.3	303	
Ethanox 702	442.4	219.1	Positive
	442.4	163.1	
Ethanox 703	264.2	219.1	Positive
	264.2	203.1	
Irgafos 126	622.3	510.2	Positive
	622.3	223	
Irganox 1010	1194.8	729.3	Positive
	1194.8	563.2	
Irganox 1076	548.5	149	Positive
	548.5	107	
Irganox 1310	296.2	167	Positive
	296.2	107	
Irganox 1330	792.6	569.4	Positive
	792.6	219.1	
Irganox 168	647.5	441	Positive
	647.5	347	
Irganox 245	604.4	263.1	Positive
	604.4	177.1	
Irganox 246	280.3	202	Positive
	280.3	77	
Irganox 259	656.5	415.2	Positive
	656.5	107	
Irganox 3114	801.6	784.5	Positive
	801.6	219.1	
MBT	168	109	Positive
	168	77	
MBTS	333	166.9	Positive
	333	123	
N,N'-(1,3-Phenylene)dimaleimide	286.1	269	Positive
	286.1	241	
Palmitic acid	255.2	255	Negative
Stearic acid	283.2	283	Negative
Tetrabutylthiuram disulphide	409.2	172.1	Positive
	409.2	116	
Thiram	241	119.9	Positive
	241	88	
Tinuvin 770	481.4	140.1	Positive
	481.4	123.1	

Results and Discussion

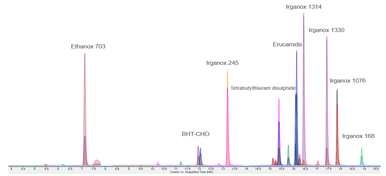


Fig 3. MRM chromatogram for 35 E&L compounds listed

Sample test result

We have tested 3 samples of rubber plugs sold in the market. With the extraction conditions described here, all samples were found to leech antioxidants, slip agents and vulkacits.

Table 2. Sample concentrations of E&Ls found in rubber plug products

Compound	Sample A (µg/kg)	Sample B (µg/kg)	Sample C (µg/kg)
Ethanox 703	14.3	ND	9.6
Disulfiram	ND	ND	98.8
BHT-OH	183.6	ND	ND
BHT-COOH	454.7	152.1	35.8
BHT-CHO	3012.6	890.8	517.4
Irganox 1310	15	101.9	3062.7
Irganox 246	ND	ND	62830
9-Octadecenamide	80.6	109.7	12866.1
Cyanox 2246	ND	41.7	140.2
Palmitic acid	16660.9	3820.8	10307.8
Stearic acid	12354.5	10506.8	9873.2
Erucamide	ND	ND	6303.8
Irganox 3114	20.6	3.6	13.4
Irganox 1010	ND	213.5	23649.7
Irganox 1330	ND	ND	12.1
Irganox 1076	329.6	11941.3	5324.5
Irganox 168	ND	ND	28481.5

Discussion

Antioxidants 1010, 1076, BHT-CHO, palmitic acid, and stearic acid exist in isopropanol at lower concentration than methanol (Table 3), so we suggest isopropanol as the dissolved solvent for extracted samples. Besides that, pipette tips also will release compounds such as Erucamide in organic solvents. It is highly recommended that clean tips with dichloromethane 2 to 3 times before pipetting.

Even with above precautions, for party of the antioxidants and slip agents, positive response can also be observed in MRM chromatogram when injecting different blanks, coupling with multiple solvent washing for needle and needle seat. It suggests those carry over response leached from rubber seal in valve system of autosampler module.

Table 3. Peak area of contaminants found in various solvent blanks

Blank Respond	BHT-CHO	Palmitic acid	Stearic acid	Erucamide	Irganox 1010	Irganox 1076	Irganox 168	Irganox 1310
Methanol	2187	2136	4625	23667	640	3911	322	426
Isopropanol	658	931	1031	79312	613	1514	217	373
2µl of air	488	770	1015	78602	587	670	235	370
No injection	362	563	709	70997	502	282	416	305

Conclusions

- 35 E&L compounds (antioxidants, slip agents, and vulkacits) were detected in rubber plug samples.
- The use of a C18 trap column placed between the Binary Pump and Autosampler is important to avoid the quantitation of false positives.
- Further precautions must be taken when considering dilution solvents, blank solvents, and lab equipment.