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THIN-LAYER CHROMATOGRAPHIC ANALYSIS OF PHENOLIC AGE RESISTERS IN ELASTOMERS IN CONTACT WITH THE BODY, DRUGS AND FOODS

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SUMMARY

Phenolic antioxidants were identified by thin-layer chromatography in 102 samples of medical and pharmaceutical rubber articles. Acetone extracts of rubber were spotted on silica gel plates with a concentrating zone. Benzene, benzene–hexane (75:25) and benzene–ethyl acetate–acetone (100:5:1) were used as developing solvents and N-chloro-2,6-dichloro-*p*-benzoquinone monoimine as spray reagent. Only 8 sterically hindered phenols among the 57 previously listed were found in current use. Reasons for choosing these compounds and the experimental conditions are discussed.

INTRODUCTION

We recently reported a thin-layer chromatographic (TLC) method developed to identify 57 phenols and 6 organic phosphites proposed as age resisters for elastomers[1]. A spray reagent which gave variously coloured spots allowed us to identify almost all of these antioxidants using only one developing solvent, although other solvents were also described in order to corroborate the results. This previous work concerned only pure substances and not real elastomer extracts. We have now applied this method to the identification of phenolic antioxidants in rubbers used in pharmacy and medicine, in order to discover which compounds among the wide range that exists are actually used. Formulations of rubbers are usually not divulged by manufacturers, which poses problems for industry and hospital pharmacists, given that the compatibility of rubbers in contact with drugs and the body can be studied easily only when the formulation of the elastomers is known[2].

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EXPERIMENTAL

The 102 articles analysed, which were produced in various countries, are listed in Table I. The rubber articles were ground to powder after having been frozen in liquid nitrogen, or simply cut into small pieces a few millimetres long using scissors. For balloon urological catheters, where several elastomers are used in the same article, the different parts were tested independently. Samples of 20 g were extracted with 250 ml of acetone for 8 h in a Soxhlet apparatus and the extracts were concentrated to about 10 ml in the last extraction cycle. In addition to this extraction pro-

TABLE I

RUBBER ARTICLES ANALYSED AND ANTIOXIDANTS IDENTIFIED

Articles	*Antioxidants identified*	*Brands*	*No. of different types*
Injection sites in intravenous administration sets for plasma, blood and solutions	III	B	4
		C	1
		D	3
		F	1
	IV	G	1
	VIII	B	1
		D	1
		E	2
		H	1
	I + II	C	2
	I + IV	A	4
		C	2
Plunger seals of disposable syringes	I	H	1
	VII	L	1
	I + III	H	1
		I	1
		J	2
		K	1
Surgeons' gloves	II	H	1
		Q	1
	VI	R	1
Stoppers and plunger seals in blood-collection devices	II	W	2
	III	X	4
Tubes of urological catheters	I	M	2
	III	O	2
	V	P	2
	VII	N	1
	I + VII	N	1
Balloons of urological catheters	I	M	2
	III	O	2
	V	P	2
	VII	N	1
	I + VII	N	1
Valves of urological catheters	II	M	2
		P	2
	III	O	2
	IV	N	2

TABLE I (*continued*)

Articles	*Antioxidants identified*	*Brands*	*No. of different types*
Rubber nipples	I	P	1
		T	2
		U	1
	II	P	1
		S	1
		U	1
Baby pacifiers	II	S	2
		V	1
Stoppers of injection vials	IV	A′	2
		B′	5
	I + IV	B′	1
Plunger seals of injection cartridges	IV	C′	4
		D′	9
		E′	6
	I + IV	E′	2

cess, commonly practised in rubber analysis[3], a second technique was also tested. The chopped-up rubber samples were collected in glass tubes with ground-glass stoppers and immersed in acetone for 24 h.

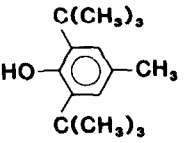
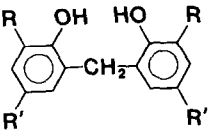
Extracts (20 μl) were analysed as in the previous study[1], with benzene as the developing solvent and N-chloro-2,6-dichloro-*p*-benzoquinone monoimine as the spray reagent, but silica gel plates with a concentrating zone (Merck 11845) were used. The R_F values and the colours of the spots were compared with those previously reported[1]. Following this, generally one, or at most two, phenolic antioxidants were assumed to be those present. Extracts were then spotted on three plates of the same type, side by side with 0.5% (w/v) acetone reference solutions of the assumed antioxidants and with mixtures of the two. The plates were developed together in light petroleum (b.p. 40–60°C) within 1 cm of the upper edge. When dry, each plate (not sprayed with reagent) was developed in one of the solvents (a) benzene, (b) benzene–hexane (75:25) or (c) benzene–ethyl acetate–acetone (100:5:1), then sprayed as above. If, in the three solvents, the R_F values and colours are identical for the samples and the reference solutions, and if the corresponding spots for the mixtures are not deformed or divided, then the antioxidants present in the rubbers are assumed to be identical with the reference compounds.

RESULTS AND DISCUSSION

Only phenolic substances and none of the organic phosphites previously studied[1] were found in the rubber articles analysed in this work. In spite of the great number of phenols proposed as antioxidants for elastomers, only the eight compounds listed in Table II were found in current use (Table I). However, given their number and variety, the rubber articles analysed constitute a valuable sample range of pharmaceutical and medical elastomers. The explanation behind this very limited

TABLE II

REFERENCE COMPOUNDS

Compounds	*Systematic and trade names [Manufacturers and suppliers (in italics) in parentheses]*	*Formula*
I	2,6-Di-*tert.*-butyl-4-methylphenol Antioxidant BHT (Bakelite; *Göbel Pfrengle*)	
II	2,2'-Methylenebis(4-methyl-6-*tert.*-butylphenol) Cyanox 2246 (Cyanamid; *Devineau*) Antioxidant OMB (Bakelite; *Göbel Pfrengle*)	 R = $C(CH_3)_3$ R' = CH_3
III	2,2'-Methylenebis(4-ethyl-6-*tert.*-butylphenol) Cyanox 425 (Cyanamid; *Devineau*)	R = $C(CH_3)_3$ R' = C_2H_5
IV	2,2'-Methylene-bis(4-methyl-6-α-methylcyclohexylphenol) Permanax WSP (Vulnax International)	R =  R' = CH_3
V	2,2'-Methylene-bis(4-methyl-6-nonylphenol) Naugawhite (Uniroyal; *Chevassus*)	R = $(CH_2)_8CH_3$* R' = CH_3
VI	Mixture of *tert.*-butyl- and *tert.*-octylcresols Wingstay T (Goodyear Chemicals; *Compagnie Française Goodyear*)	
VII	Styrenated phenol Naugard SP (Uniroyal; *Chevassus*)	
VIII	Sterically hindered bis-phenol Vulkanox CS (Bayer; *Bayer-France*)	

* Formula deduced from the chemical name (these names are sometimes unduly simplified by the manufacturers when the chains are branched).

** These spots appear only after heating the plates at 100°C in an oven.

choice apparently does not lie in regulations imposed for toxicological reasons. The articles analysed came from various countries, including several where no regulations govern this question. In such cases, manufacturers generally use compounds approved for alimentary contact. All the phenolic substances listed in our previous work[1] are in fact approved for such use, sometimes with limited concentrations.

Criteria determining the choice of antioxidants for rubbers have been reviewed

Approximate R_F × 100 values with light petroleum, then			*Colour of spots*
Solvent a	*Solvent b*	*Solvent c*	
86	86	85	Yellow
65	48	72	Yellow
70	53	73	Yellow, green edge
74	55	76	Yellow
70	53	82	Yellow, green edge
80	83	84	Yellow, green edge
63**	48**	68**	Purple
72	64	75	Violet
89	90	91	Pink
36	18	57	Blue
71		61	Grey
72	56	81	Violet blue
23	11	49	Cream

elsewhere[4]: some ageing factors are external (*e.g.*, oxygen, ozone, heat and light) and others are internal (*e.g.*, nature of the polymer, vulcanizing system and degree of vulcanization). In addition to the absence of toxicity, it was possible to assume that the nature and the use of a pharmaceutical or medical article, and also the sterilization process, when it is required, represented some of the most important criteria of choice. In fact, the same antioxidants were found in several articles used for very

different purposes. For instance, urological catheters of brand O and injection sites (junctions) of intravenous administration sets of brands B, C, D and F (catheters and sets sterilized with ethylene oxide) contain antioxidant III. Hence the nature and the use of an article have no or little effect on the manufacturer's choice of antioxidant. Compounds other than I and IV, however, were not found in rubber stoppers for injection vials or in plunger seals of injection cartridges, in spite of the large number of these samples. Surgeons' gloves sterilized with ethylene oxide (brand H) or by irradiation (brand Q) contain the same antioxidant (compound II). Other articles also may contain the same antioxidant whatever the sterilization process used. For example, plunger seals of brand L syringes, sterilized with ethylene oxide, and tubes of brand N urological catheters, sterilized by irradiation, contain compound VII; injection sites in brand G infusion sets, sterilized with ethylene oxide, and brand N catheter valves, sterilized by irradiation, contain compound IV. Rubber nipples and baby pacifiers, which are not sterilized by the manufacturer but only by the user, using boiling water or by autoclaving, may contain the same compounds as articles sterilized with ethylene oxide. Indeed, all baby pacifiers and some rubber nipples (brands P, S and U) contain compound II, which was also found in infusion set junctions (brand C), in urological catheter valves (brands M and P), and in gloves (brand H). Some other nipples (brands P, T and U) contain compound I, which is also used in injection sites of infusion sets (brands A and C), in syringe plunger seals (brands H, I, J and K) and in catheter tubes and balloons (brand M). Compound I was also found in some catheter tubes and balloons (brand N) sterilized by irradiation. Hence antioxidants do not seem to be chosen on the basis of the sterilization process. Compounds other than I and II were not found, however, in rubber nipples and pacifiers. However, this may be due to the smaller number of samples of these two articles.

Further, the nature of the antioxidant does not depend on the brand, as different articles of the same brand (*e.g.*, brand H gloves and syringe plunger seals and brand P nipples and urological catheters) do not contain the same phenolic compound. Even more remarkable is the case of different types of the same article of the same brand, such as the injection sites of the different types of infusion sets of brands B, C or D, in which different phenols were found. In the balloon urological catheters analysed, the same antioxidant is used in the tubes and in the balloons, but another compound is present in the valves. For brand O, however, compound III is used in the three parts.

Most of the antioxidants identified are sterically hindered molecules, which results in a very low volatility[4]. Compound II, for instance, is generally considered as one of the best phenolic antioxidants. It has recently been shown[5] that compound I, when irradiated, may change into its dimer and trimer. Given that unchanged compound I was found to be present in the rubbers analysed, this change, if it occurred, was not complete.

Another interesting fact is that very often the same compound is used in articles having the same colour and the same macroscopic shape. Injection sites in brand A and C infusion sets, for example, contain compounds I and IV and have the same colour and shape. Those of brands B, C, D and F, all of which contain compound III, also have identical colours and shapes. The link between aspect and antioxidant (and probably between aspect and all ingredients if they had been analysed) clearly

shows that these rubber junctions come from the same manufacturers, even if the infusion set brands differ. Thus, the infusion set manufacturers only assemble the junctions but do not make them.

From an analytical point of view, grinding of rubbers and extraction in a Soxhlet apparatus are not absolutely necessary. Indeed, good results are also obtained by cutting the rubber articles into small pieces and then extracting the antioxidants by soaking. Such an analysis may therefore be carried out without specialized equipment. This simplified extraction method may be even more advantageous given that extraction is not exhaustive and thus chromatograms are easier to interpret. However, rubbers are sometimes composed of mixtures of several elastomeric polymers in very different proportions, each of these elastomers having a different antioxidant. Some cases where two antioxidants were found (compound I always present) may correspond to such mixtures. The volume of sample spotted may have to be increased if a spot obtained is not clearly visible because of the small proportion of the polymer stabilized with the corresponding compound.

The first chromatographic development in light petroleum was proposed[6] to remove oils and similar low-polarity materials found in rubbers, because they can cause streaking of the spots and may obscure other high R_F components; light petroleum carries the oils near to the solvent front and out of the way of other rubber ingredients. In addition, we observed that antioxidant R_F values were lower for rubber extracts and for their mixtures with reference substances than for pure compounds. This inconvenience may be overcome by first developing in light petroleum. This is particularly useful with antioxidants II and IV. If this development is not done, antioxidant IV in a rubber extract has an $R_F \times 100$ value of 65 (Fig. 1a), instead of 74 (Fig. 1b), *i.e.*, equal to that of antioxidant II, spotted as a reference

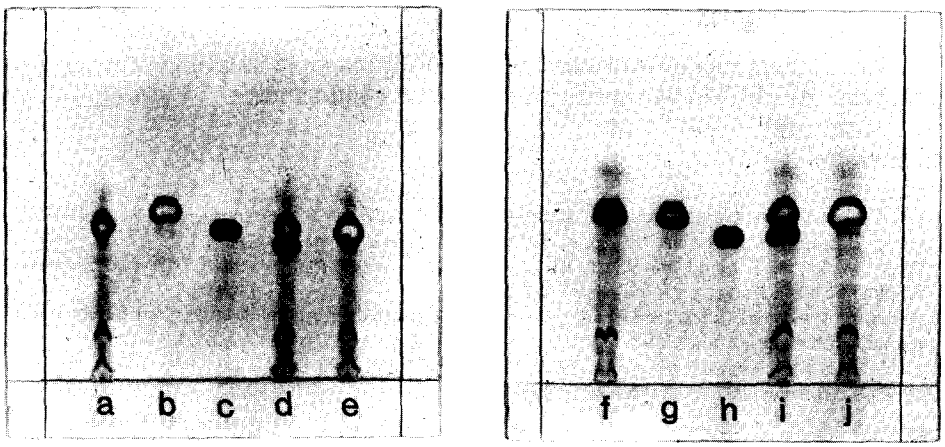


Fig. 1. Chromatograms without (a, b, c, d, e) and with (f, g, h, i, j) preliminary development in light petroleum. a, f, Rubber extract containing antioxidant IV; b, g, antioxidant IV as reference compound; c, h, antioxidant II as reference compound; d, i, mixture of the rubber extract (containing antioxidant IV) and antioxidant II (as reference compound); e, j, mixture of the rubber extract (containing antioxidant IV) and antioxidant IV (as reference compound). Migration of the antioxidants in the presence of extract (a, d, e, f, i, j) is delayed by the oils when no preliminary development in light petroleum is applied.

(Fig. 1c), although the tests were carried out side by side on the same plate. Given that compounds II and IV have similar chemical structures and thus show identical colours with the spray reagent[1], their confusion is inevitable. When compounds II and IV are mixed with rubber extracts (Fig. 1d and e), their R_F values, however, are also decreased. However, preliminary development in light petroleum allows an immediate correct interpretation (Fig. 1f and j). Except for these two compounds, the antioxidants ultimately identified are those expected on the basis of results obtained from chromatography in benzene, although it was carried out without reference substances and the assumptions made only on the basis of results obtained previously[1]. This, associated with the large number of possible compounds, emphasizes the interest in a spray reagent giving a wide variety in spot colours. It follows that, when manufacturers monitor the formulation of their products, preliminary development in light petroleum may generally be omitted, provided that antioxidants II and IV are not involved. Indeed, during such monitoring, the identity of the antioxidants is known and what is in question is only its verification.

CONCLUSION

This study led us to conclude that very few phenolic compounds are actually used in pharmaceutical and medical rubbers, in spite of the wide variety available, and that their choice does not depend on the sterilization processes or on use or brand of articles. In addition, the results validate the method we had previously proposed and indicate that such analyses can be carried out without sophisticated equipment. This should induce all those concerned with problems of rubber quality control and elastomer compatibility, be they rubber or drug manufacturers or hospital pharmacists, to attempt to solve them.

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