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CHREV. 133

QUANTITATION OF NON-HALOGENATED COMPOUNDS BY ELECTRON-CAPTURE GAS CHROMATOGRAPHY

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CONTENTS

1. INTRODUCTION

The electron-capture detector (ECD) has been used in gas chromatography (GC) for quantitative purposes for about 15 years. The high sensitivity combined with the selectivity that can be achieved are the reasons why this device has been used so much in the analysis of organic compounds at the trace level in various complex sample types.

This review considers the possibilities of using the ECD in the analysis of non-halogenated compounds that do not contain a nitro group. The introduction of halogenated groups in derivatization reactions will not be considered as such information has been included in other reviews[1–3]. Surveys on the use of electron-capture detection in GC have been published[4–6].

The inventor of the ECD, J. E. Lovelock, ranked various compounds in order of relative absorption coefficients for thermal electrons and proposed the expression "electrophore" for those atoms or structures in a molecule which confer electron-absorbing properties or electron affinity to the molecule[7,8]. Two classes could be distinguished, of which halogen and nitro substituents belong to the simple electrophores. The other class consists of conjugate electrophores, which are found in structures with certain groups which when isolated do not contribute to electron capture, but do so if connected by specific bridges. Typical conjugate electrophores are carbonyl compounds such as biacetyl, fumarate esters, cinnamaldehyde and quinone. Fumaronitrile and azobenzene represent other structures of this kind.

The electron-capture process has been suggested to occur according to two different mechanisms[7–9]. The first is characteristic of several conjugate electrophores and is called the resonance capture or non-dissociative mechanism and results in the formation of a negative molecular ion. The second type is found among halogenated

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compounds such as pesticides and is called the dissociative mechanism as electron attachment leads immediately to the formation of a negative ion (*e.g.*, a halide ion) and a neutral radical. The negative molecular ion formed upon electron attachment is best detected at low detector temperatures as electron detachment from this ion is favoured by high temperatures. The temperature dependence is related to the overall structure of the molecule and it is of considerable importance for the analyst to be able to determine this effect as the relative response can vary by more than 1000-fold over the useful detector temperature range (100–350 °C). Response factors without data on detection temperatures are of limited value[10,11]. It is also important to remember that the temperature of the detection zone is not necessarily the same as that indicated for the detector oven.

The response of the ECD is also influenced by the mode of operation. Modern constant-current ECDs usually collect the electrons via an applied pulsed voltage, which gives better linearity and fewer disturbances than older types operating in the non-pulsed mode. The latter type has been shown in some studies with compounds responding in the non-dissociative mode to have a less pronounced temperature dependence than in the pulsed mode. This was the case for benzophenone and dibutyl phthalate[12]. However, there were also differences between the same type of instrument. From a theoretical point of view the pulsed mode is the one of choice, as pointed out by Wentworth and co-workers[10,13].

Most compounds of interest to the analyst do not capture electrons with thermal energy, which is the reason for the selective response of the detector. Some structures are discussed below that have been quantified by electron-capture detection. They represent mainly the group of conjugate electrophores.

2. CONJUGATED CARBONYL GROUPS

Several reports have described the quantitative use of electrophores where a carbonyl group is involved.

TABLE 1

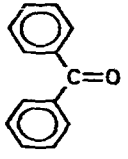
MINIMUM DETECTABLE CONCENTRATION (MDC) OF VARIOUS COMPOUNDS[14,15]

Compound	*MDC (moles/sec·10^{15})*	*Temperature (°C)*	*Mode*
Benzophenone	1.2–3.1	104–163	d.c.
p-Chlorobenzophenone	3.8	104–163	d.c.
2-Benzoylpyridine	4.8	104–163	d.c.
2-(4-Chlorobenzoyl)pyridine	1.2	104–163	d.c.
Benzoylfuran	7.3	170	d.c.
Benzoylthiophene	6.4	170	d.c.
Anthraquinone	4.7	200	d.c.
Dibenzosuberone	5.0	200	d.c.
Di(ethylhexyl) phthalate	52	220	d.c.
8-Methoxypsoralen	2	200	d.c.
Methindione	0.4	215	d.c.
Saccharin	0.3	210	d.c.
Sulfapyridine	0.8	350	Pulsed
N_4-Acetylsulfapyridine	1.2	350	Pulsed

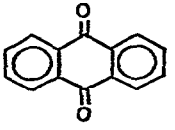
Benzophenone (I) is a typical conjugate electrophore and has a pronounced temperature-dependent response. This seems also to be related to the type of detector, as devices with a pulsed voltage exhibit a stronger decrease with increasing temperature than those of the direct-current type[12]. The response for benzophenone is comparable to that of many other well known halogenated electrophores[14] (*cf.*, Table 1). It is also of interest that at low temperatures *p*-chlorobenzophenone is less sensitive than benzophenone, which indicates the importance of the conjugated system. At higher temperatures the chloro substituent takes over the electron attachment and *p*-chlorobenzophenone becomes the more sensitive compound.

The relative responses for a series of benzophenones have been reported[14]. Electron-donating, non-electrophore substituents gave an increased response. Some of the benzophenones were used in the quantitation of drugs, which upon oxidation formed the corresponding benzophenones[16].

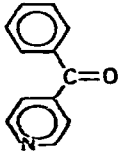
A similar approach was used for the antidepressant drug amitriptyline, which after oxidation gave anthraquinone (II). From the temperature dependence, the electron-capture mechanism was stated to be of a dissociative nature[17]. This was also found by Grimsrud[18] in studies involving also halogenated hydrocarbons and anthracene.



I

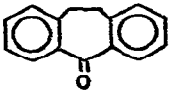


II

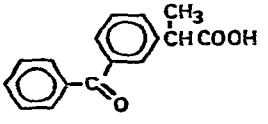


III

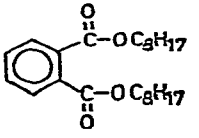
Benzoylpyridine (III) has about the same response as benzophenone[14]. This was shown to be true also for benzoylfuran and benzoylthiophene, which were obtained by benzoylation of furan and thiophene in order to confer upon them electrophore properties[19]. The response of dibenzosuberone (IV) was in the same range[17] (Table 1). The benzophenone moiety of ketoprofen (V) made possible its determination in biological fluids[20].



IV



V

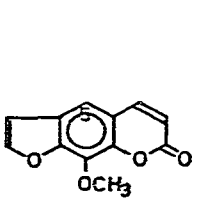


VI

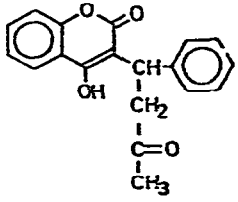
The analysis of phthalates (VI) in various sample types has been of great interest. The conjugate electrophore gives the molecule reasonably good sensitivity, which can be used in trace analysis[21]. In a given situation the selectivity can be good, as was demonstrated in the search for di(ethylhexyl)phthalate (DEHP) in blood plasma[22]. On one occasion a complicated pattern obtained with the ECD was shown to be the same as in gas chromatography–mass spectrometry when the characteristic fragment *m/e* 149 for phthalate was monitored.

Phthalate esters can make considerable contributions to the contaminant pattern observed with the ECD from organic solvents and plastic devices used in the analytical procedure. If the ECD response for the compound to be analysed exhibits a temperature response that differs from that of the phthalates, *i.e.*, shows an increase with increasing detector temperature, their interference can be diminished. Even for the phthalates the temperature dependence was more pronounced in a detector operating in the pulsed mode than in the d.c. mode[12]. A non-dissociative mechanism was indicated for DEHP from the temperature dependence found in a pulsed detector[23]. A study of the relative response for some phthalates at 230, 290 and 320 °C was recently reported[24]. Diallyl phthalate was the only ester which did not lose sensitivity at the higher temperature. The samples included methyl and ethyl benzoates, which as expected were a factor of 100–1000 times less sensitive.

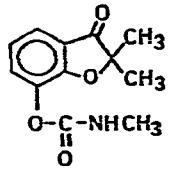
8-Methoxypsoralen (VII) is of importance in the treatment of psoriasis. It has been shown to be a good electrophore, permitting the analysis of nanogram amounts in biological fluids[25]. The temperature-dependent response indicated a non-dissociative mechanism as the detector response at 200 °C was about 30 times higher than that at 300°C. It was interesting that the response for the isomer 5-methoxypsoralen was only slightly temperature dependent, which shows that minor structural changes can influence the electron-capture mechanism.



VI



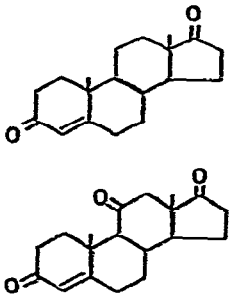
VII



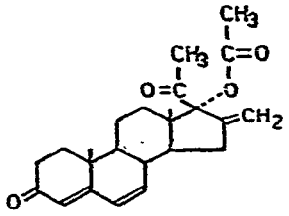
IX

A compound containing a similar structural feature and also of considerable therapeutic interest is warfarin (VIII). This drug can be derivatized with electrophores such as pentafluorobenzyl bromide via the phenolic function, but a technically less complicated procedure makes use of diazomethane, which from an ECD point of view is "transparent", to form the methyl ether. With the detector temperature kept at 300°C the methylation procedure permitted the analysis of 100 ng/g in various tissues[26]. The insecticide carbofuran has one metabolite (IX) with a keto group, which can be quantitated by GC–ECD[27]. The conjugate electrophore in this instance involves only a carbonyl group in an α-position, which as an electrophore could be compared with acetophenone or benzaldehyde. However, the presence of a carbamate group in oxo-carbofuran might have an auxiliary function. The advantage of direct analysis instead of derivatizing the carbamate group was stressed. Although a relatively high detection temperature (275°C) was used, the procedure permitted the detection of *ca.* 100 pg on the column.

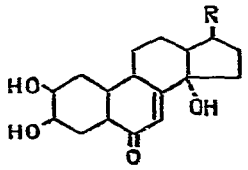
Lovelock *et al.*[28] pointed out the possibility of detecting selectively some steroids with unsaturated ketone structures, such as 4-androstene-3,17-dione (X). The 4-ene-3-one system was indispensible for good detector response. 4-Androstene-3,11, 17-trione was the most sensitive compound (X). Melengestrol acetate (XI) has been



X



XI

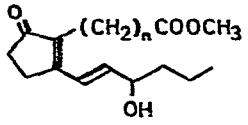


XII

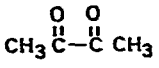
determined using GC–ECD[29]. The ECD response was comparable to that for the most sensitive haloesters of steroid alcohols. The structural requirements for the high response of this compound type were studied by Koshy[30]. Among the important functional groups were 17α-acetate and substitution with 16-methyl and a 6-keto group. The high ECD response was independent of temperature between 260 and 360 °C and permitted 10 pg to be determined.

A similar structural feature can be found in some polyhydroxy steroids, the ecdysones (XII), which act as insect-moulting hormones. Poole and Morgan[31] found that the electrophore was an unsaturated ketone structure (7-ene-6-one), with important contributions from a hydroxy substituent in position 14, either free or preferably silylated. The temperature dependence of the detector response was indicative of a non-dissociative mechanism. For practical reasons a temperature of 300°C was used in the detector, but 5 pg of the TMS ethers could be detected.

The presence of this structural element, an unsaturated ketone, has made possible the determination, after silylation, of some prostaglandins B (XIII), which are dehydration products of prostaglandins E[32]. The latter did not respond in the ECD.

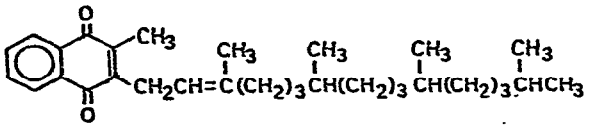


XIII

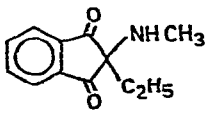


XIV

Of the various electrophores mentioned in Lovelock's early work, the ECD has also been used for the quantitation of biacetyl (XIV) and pentane-2,3-dione in beer[33]. In other sample types quinone derivatives such as vitamin K (XV) have been studied[34]. The compounds required high temperatures for elution, which fixed the detector temperature at a high value (305°C).

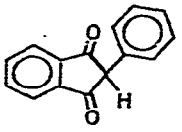


XV

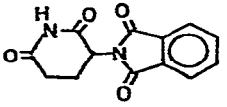


XVI

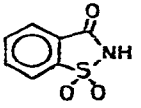
A structure similar to the phthalate electrophore is present in methindione (XVI), which can be detected at the low picogram level[35]. This compound did not show a temperature-dependent response in a non-pulsed detector over the range 150–215 °C, which indicates either a very stable negative molecular ion or a dissociative mechanism. This structure of conjugated β-ketones is present in some other drugs, *e.g.*, phenindione (XVII), which was shown to have comparable sensitivity after masking of the active hydrogen[35]. Another related structure is present in thalidomide (XVIII), which was reported by Zlatkis and Lovelock[8] to have an electron-capture coefficient of *ca.* 300 (compared to chlorobenzene). The drug was withdrawn from the market before interest in trace analysis by GC–ECD had begun, otherwise it would have been possible to devise a selective and sensitive method.



XVI



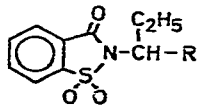
XVII



XIX

Saccharin (XIX) can also be placed in the group of conjugated carbonyl electrophores. This compound has been analysed with an ECD in various biological samples[36]. To obtain the best detectability, the acidic sulphonamide function has to be alkylated, preferably in an extractive alkylation reaction[37]. The sensitivity was such that 2 pg could be detected.

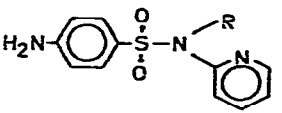
The saccharin-like structure has been used in the analysis of a drug candidate (XX, 1), which before GC had to be converted into a less polar structure (XX, 2) to reduce its column adsorptive properties. This change was achieved in a dehydration reaction with trifluoroacetic anhydride without increasing the chromatographic background[38].



1) R = $CONH_2$

2) R = $C{\equiv}N$

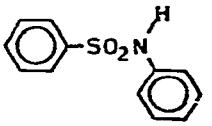
XX



1) R = H

2) R = CH_3

XXI



XXII

3. SULPHONAMIDES

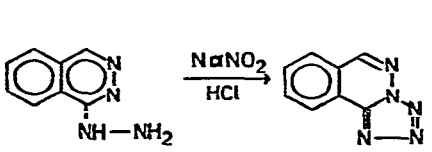
Saccharin can be regarded as a special form of a sulphonamide. It has recently been shown, however, that even conventional sulphonamides of therapeutic interest, *e.g.*, sulfapyridine (XXI, 1) can be gas chromatographed and quantified after either alkylation or acylation[39]. The best sensitivity was obtained after alkylation with

pentafluorobenzyl bromide and acylation with heptafluorobutyric anhydride. Of great interest was the observation that both alkylation with a methyl halide (XXI, 2) and acylation with acetic anhydride resulted in derivatives with a sensitivity not much lower than that of the fluorinated derivatives. This means that under certain conditions the sulphonamide group is acting as an electrophore[39]. The conclusion was that the sulphonamide group should be attached to two aromatic rings (delocalized systems) in order to obtain the highest response. The temperature dependence of the detector response has been studied for some model compounds, and for the one most similar to drugs, N-phenylbenzenesulphonamide (XXII), methylation and acetylation gave derivatives that were about 10 times less sensitive at 350°C than at 150°C[40]. The possibility of using reagents that usually give derivatives transparent in the ECD (such as acetic anhydride) or which are volatile (such as the methyl halides) adds considerably to the advantage of using an ECD for a compound with inherent electrophore properties.

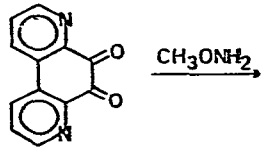
4. MISCELLANEOUS COMPOUNDS

Very early it was found that hydrocarbons are more or less transparent in the ECD. However, there are exceptions among the polycyclic aromatic hydrocarbons, such as anthracene and benzanthracene[41]. It is also of interest that azulene and cyclooctatetraene give a strong response. In the latter instance this was explained as being due to the favourable stabilization of the planar negative ion.

A polycyclic heteroaromatic compound having good electrophore properties has been reported for a reaction product (XXIII) between the antihypertensive agent hydralazine and nitrite[42]. Hydralazine, which is labile, was trapped in the biological sample from which the product was isolated and then quantitated in nanogram amounts with an ECD.



XXIII

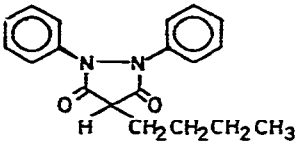


1 XXIV 2

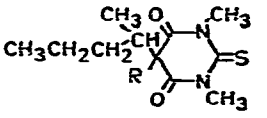
Another polycyclic compound, phanquone (XXIV, 1), has also been isolated after derivatization in the aqueous phase and then subjected to electron-capture detection[43]. Phanquone was reacted with methoxylamine to give the bismethoxime derivative (XXIV, 2), which was detected with a pulsed ^{63}Ni ECD at 300°C. A concentration of 15 ng/ml could be quantified in biological fluids.

An example of a structure that would not directly be considered as an electrophore is phenylbutazone (XXV), a widely used anti-inflammatory drug. A recent paper nevertheless described the use of an ECD for its selective determination in biological fluids[44]. Concentrations of 10 ng/ml could be quantified accurately and very clean chromatograms were obtained. Sulphinpyrazone has been determined by GC–ECD, the compound undergoing an elimination reaction to give an analogue of phenylbutazone[45].

Although free barbituric acids were reported to have some electron-capture response, no applications have been described until recently. The methylated derivatives of thiopental and thioamytal (XXVI) were studied[46] and at a detector temperature of 300°C, 0.1 and 1 ng, respectively, could be detected.



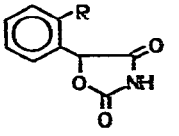
XXV



1) R = C_2H_5

2) R = $CH_2CH{=}CH_2$

XXVI



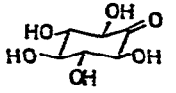
1) R = H

2) R = CH_3

XXVII

A structure in part related to the barbiturates is that of pemolinedione (XXVII,1), produced by acid treatment of pemoline, a drug which is active at the micrograms per millilitre level. Owing to the selective response a very simple assay was developed with XXVII, 2 as internal standard[47]. The response was such that 0.5 ng injected could be detected (detector temperature 230°C, d.c. mode).

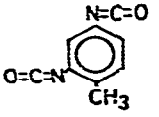
A compound that has been reported to have a high electron affinity as the silyl derivative is myo-inosose-2 (XXVIII)[48]. In a pulsed detector at 205°C a few picograms could be detected. Whereas the corresponding inositol was 500 times less sensitive, it was found that one form of fructose was 3–4 times more sensitive. Although the non-conjugated carbonyl group is primarily responsible for the high affinity, the structure of the species that gives the compound the electrophore properties is probably different.



XXVIII

NCS—CH_2CH_2—SCN

XXIX



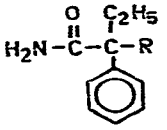
XXX

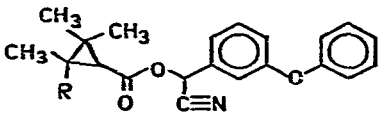
Ethylene bis(isothiocyanate) (XXIX) is another type of structure that has been quantified with the ECD. At a detector temperature of 300°C 0.02 ppm could be determined[49]. The vicinal position of the isothiocyanate groups might be the reason for the good response in a similar manner to that of bis(1,2-trifluoroacetyl) glycols[50]. A related substituent is found in toluene 2,4-diisocyanate (XXX), which responded well in the ECD[51]. Some compounds with related structures did not give a response.

The correlation between chemical structure and electron-capture response has been studied with some isothiocyanates and isocyanates[52].

Phenylethylmalonamide (XXXI) is a metabolite of primidone. A GC–ECD method has been reported in which the compound was dehydrated with trifluoro-

acetic anhydride in a similar manner to the reaction described above for the saccharin derivative (XX, 1). Only one of the primary amide groups was converted into a nitrile[53]. This derivative had sufficient response to allow the determination of submicrogram amounts per millilitre. The electrophore component in this compound is not clear.





1) R = $CONH_2$

2) R = $C{\equiv}N$

XXXI

1) R= CH_3

2) R= $CH{=}CCl_2$

XXXII

An unexpected response was shown for one pyrethroid insecticide (XXXII, 1). The electrophore in this compound is not clear[54]. Two related compounds contained a dichlorovinyl substituent instead of a methyl group, but their sensitivities were approximately the same, *i.e.*, around 5 pg injected on-column with a detector temperature of 270°C. This implies that there is an inherently very good electrophore in the structure.

5. OTHER ASPECTS

In connection with these various compounds it should be mentioned that, apart from contamination of solvents with phthalates, impurities due to photochemical oxidation of hexane isomers have been reported[55]. The structure of these electrophore contaminants has not been elucidated, but it shows the importance of limiting the presence of unknown electrophores by careful handling and storage of chemicals.

The previous discussion has shown that various structures can be detected and quantified with an ECD. The importance of the conjugated structure in benzophenone and *p*-chlorobenzophenone is best shown by their almost identical responses at low temperatures. There must, certainly, be other structures present in which the halogen content is not the important component in the electrophore structure. This may be the case among many members of the benzodiazepine group, which have a well conjugated system[6]. Other interesting structures from that point of view are indomethacin[56] and griseofulvin[57]. The fact that the dominant electrophore is not always a halogen is still overlooked.

Zlatkis and Lovelock[8] stated in 1965 that the difficulty in predicting the response of conjugate electrophores was related to the fact that the structure one should consider is that of the resultant negative ion, not the parent molecule. In some instances it is now possible to elucidate the structure of negative ions formed under electron-capture conditions. By use of atmospheric pressure ionization mass spectrometry (API-MS), Horning *et al.*[58] have found the expected structure for, *e.g.*, benzil.

One benzodiazepine, flunitrazepam, was shown to undergo a rearrangement[59]. Some conditions in the detector during electron capture might have greater importance than it had earlier been reasonable to believe. The influence of trace amounts of oxygen and water is thus considerable for the detection of some electrophores[18]. Anthracene showed an increased response with very low levels of oxygen, while some halohydrocarbons were affected only at high levels. For anthracene this effect was greatest at high temperature. An exchange reaction between the negative molecular ion and oxygen was proposed. This suggests that control of the carrier gas contamination might be important in order to obtain the best and most reproducible results. In this respect anthraquinone was affected like the halogenated hydrocarbons[18].

In connection with this discussion, it is also of interest that so far no negative ion has been found for benzophenone in the API-MS instrument[60].

The recent availability of negative ion mass spectrometry has opened up interesting possibilities both for the application of electron-capture processes of compounds that form stable negative ions and also for obtaining an insight into the structures formed. The conditions for the formation of electrons with thermal energy are, however, not equivalent in GC–ECD and in electron-capture negative ion GC–MS[61]. In this respect the API instrumentation is more suitable.

5.1. Quantitation

In the analysis of trace amounts of organic compounds by gas-phase methods, the use of internal standards has found widespread use. The internal standard must be chosen with care in order to optimize the procedure. The best approach is now considered to be the use of analogues, homologues or isomers. However, it is as important to know the properties of the internal standard as those of the compound to be analysed. In connection with electron-capture detection, this means that the mechanism or at least the temperature dependence of the detector response has to be evaluated. Of the compounds covered in this review, examples of differences in this respect can be found among the benzophenones and the alkoxypsoralens. The use of a halogenated compound, *i.e.*, a pesticide that captures thermal electrons only according to a dissociative mechanism, cannot fully compensate for variations in the operating conditions in the same way as an analogue. Examples of ideal internal standards for ECD purposes can be found in papers on the determination of methindione[35], methoxypsoralen[25], di(ethylhexyl) phthalate[22], pemoline[47], phenylethylmalonamide[53], sulfapyridine[39] and a saccharin derivative[38].

The analysis of compounds with inherent electrophore properties can be very simple even if a derivatization reaction has to be used to mask polar functional groups. In comparison with reactions for the introduction of electrophores such as heptafluorobutyryl this makes a large difference, as the latter derivatization products are often accompanied by products from other substrates in the sample extract.

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7. SUMMARY

The use of electron-capture gas chromatography for the quantitation of non-halogenated compounds that do not contain a nitro group is reviewed. The electrophore most often used is the conjugated carbonyl group; about fifteen examples are given on selective and sensitive determinations. Sulphonamide-containing compounds represent another interesting group useful for quantitative work. Finally some electrophore compounds belonging to various classes are mentioned.

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