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Comparison of Drug Retention Indices Determined on Packed, Wide Bore Capillary and Narrow Bore Capillary Columns

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ABSTRACT: The retention indices of 75 drugs and other compounds of toxicological significance have been measured on SE-30 equivalent, chemically bonded, fused silica capillary columns under isothermal conditions. The data have been assessed to determine the validity of using retention indices measured on packed columns for the identification of compounds eluting from narrow bore thin film or wide bore thick film capillary columns. The results indicate that the extensive retention index data bases published for SE-30 packed columns may be used for the preliminary identification of peaks eluting from capillary columns in toxicological screening. The study also indicates that retention index data bases generated on narrow bore capillary columns are applicable to wide bore thick film capillary columns.

KEYWORDS: toxicology, drug retention indices, chromatographic analysis

Gas chromatography (GC) is one of the most widely used techniques in analytical toxicology for the identification and quantification of compounds. The identification of an unknown compound is made by comparing its retention properties (retention index, RI) with those of reference samples recorded in a data base. Packed columns have generally been favored by toxicologists until recently, when a number of major technological advances have made capillary gas chromatography more robust and easier to use. The most important changes have involved the introduction of inert flexible fused silica columns (inside diameter [ID] $\simeq$ 0.2 mm) and chemically bonded stationary phases to replace glass columns with nonbonded wall coated stationary phases [*1*]. These new columns retain high efficiencies, can be washed free of contaminants, and can be prepared with increased film thicknesses while their flexibility makes them easy to install in GC equipment. More recently, wide bore (0.53-mm ID) fused silica capillary tubing has been manufactured and used to prepare columns with chemically bonded thick films. These "wide bore" or "megabore" capillary columns are regarded as the "capillary replacement" for packed columns and provide an easy way to use fused silica column technology with packed column GC equipment.

Several data bases have been compiled for toxicological work giving retention indices on

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SE-30 packed columns for drugs and poisons likely to be encountered in forensic science samples [2–7], that of Ardrey et al. [2] being the most extensive. The rapid changes in GC column technology have meant that laboratories have been unable to generate and update continually data collections on every type of capillary column. Consequently, a few studies have been carried out already to determine whether existing data bases on packed columns may be used with various types of capillary column.

Comparison of retention properties for drugs on nonbonded, SE-30 equivalent wall coated open tubular columns (WCOT) (ID $\simeq$ 0.2 mm) with packed column data showed marked differences [8,9], and Schepers et al. [8] concluded that it was unwise to carry out analyses on these nonbonded capillary columns with the subsequent use of packed column data for identification. Differences were also found to occur between WCOT columns produced by different manufacturers [8,9]. The introduction of chemically bonded fused silica capillary columns again raised the question of the validity of transferring retention index data between columns. Perrigo et al. [10] compared retention data generated on narrow bore columns of this type with packed column data generated in their own laboratory and found the data to be highly correlated although some compounds showed marked deviations. Comparisons of narrow bore chemically bonded columns (SE-30) with nonbonded columns from the same manufacturer showed relatively good agreement but again some differences occurred [10,11]. Subsequently, data bases of retention indices for drugs and poisons using SE-30 chemically bonded narrow bore columns from various manufacturers have recently appeared in the literature [11–16].

The introduction of wide bore, chemically bonded fused silica columns with thick films and their potential use in analytical toxicology has indicated that the retention properties of these columns also need to be examined. Therefore, in this paper, an assessment is made on the validity of transferring isothermal retention indices between wide bore thick film capillary columns, narrow bore thin film capillary columns, and conventional packed columns all having the low polarity SE-30-type stationary phase. This phase has been shown to be the most suitable for toxicological analysis [3,17] and has been used widely in U.K. forensic science laboratories. It is also the stationary phase recommended for international comparisons because of its extensive worldwide use [2].

Experimental Procedure

Materials

Pentane and *n*-butyl acetate were AnalaR grade from BDH (Poole, Great Britain). All drug samples were from the drug collection of the Central Research Establishment, Home Office Forensic Science Service. Straight chain alkane hydrocarbons were obtained from SGE (Milton Keynes, Great Britain) and Sigma (Poole, Dorset, Great Britain).

Gas Chromatography

Chromatography was performed using a Perkin Elmer Sigma 3B gas chromatograph interfaced to a Trivector Data Station (Trivector Scientific Ltd., Sandy, Great Britain) for data handling. A capillary injector operated in the split mode at a split ratio of 20:1 was used for the narrow bore (0.22-mm ID) columns, whereas for the wide bore columns (0.53-mm ID), a 1/4-in. (0.6-cm) packed column injection port fitted with a J & W inlet adaptor and glass liner for direct flash vaporization injection was employed (Jones Chromatography Limited, Glamorgan, Great Britain). The carrier gas was nitrogen operated at flow rates of 1 and 10 mL/min for narrow bore and wide bore columns, respectively. Flame ionization detection was used.

Solutions of straight chain hydrocarbons in pentane were prepared as references for the calculation of retention indices [*18*]. Isothermal RI values were measured at oven temperatures of 130°C for compounds with RI values up to 1600, 200°C for compounds with RI values between 1600 and 2200, and 275°C for compounds with RI values 2200 to 3300. Drugs, either as free acid or free base, were dissolved in *n*-butyl acetate at concentrations of 50 or 100 ng/μL (for narrow bore columns) and 50, 100, or 500 ng/μL (for wide bore columns). The higher concentrations were selected for late eluting compounds, for drugs having a poor detector response, and for some acidic compounds. Aliquots of 1 μL were injected in the gas chromatograph using the hot needle technique [*19,20*]. All retention index measurements were carried out in duplicate. The injector temperature was 280°C throughout, whereas detector temperatures were 180, 250, and 325°C, respectively, for the three oven temperatures.

Three types of fused silica nonpolar bonded phase capillary columns were used for RI measurements:

(1) BP1 narrow bore, 25-m by 0.22-mm ID with 0.25-μm film thickness (SGE, Milton Keynes, Great Britain),

(2) BP1 wide bore, 25-m by 0.53-mm ID with 1.0-μm film thickness (SGE, Milton Keynes, Great Britain), and

(3) DB1 wide bore, 30-m by 0.53-mm ID with 1.5-μm film thickness (J & W Scientific, Jones Chromatography Limited, Glamorgan, Great Britain).

Results and Discussion

The isothermal retention indices of 75 drugs determined on the bonded phase fused silica capillary columns are presented in Table 1, arranged in ascending order of elution on the narrow bore column. The same data are listed alphabetically by compound name in Table 2. The packed column data also shown in the tables have been abstracted from the data base prepared by Ardrey et al. [*2*]. The compounds in this study were selected to cover a wide range of different chemical and pharmacological classes of drugs which are commonly encountered in toxicology casework. Included in the compounds selected were some for which reliable retention data were available on SE-30 packed columns (that is, RI values measured in 10 or more laboratories gave good agreement [*4*]). Others were deliberately chosen because RI values previously reported on SE-30 bonded [*10,12,16*] and nonbonded [*8,21*] capillary columns did not correlate well with SE-30 packed column data or gave multiple peaks.

The correlation coefficients (r) for all pairs of columns examined are presented in Table 3 clearly demonstrating that all pairs are highly correlated. All data points available have been used to calculate the correlation coefficient for each pair of columns. When calculating the correlation coefficients, the retention index of the major peak was used for drugs giving multiple peaks in the chromatogram. Some of the results are illustrated graphically, showing the correlation between narrow bore capillary and SE-30 packed columns ($r = 0.9966$) in Fig. 1, wide bore capillary (SGE) and SE-30 packed columns ($r = 0.9952$) in Fig. 2, and narrow bore and wide bore (SGE) capillary columns ($r = 0.9994$) in Fig. 3. The wide bore columns obtained from two different manufacturers (SGE and J & W) were also highly correlated ($r = 0.9967$).

The interlaboratory standard deviation of retention index measurements is 15 to 20 RI units [*3*] so that a search window of $\pm$50 to 60 RI units has often been used when comparing the RI of an unknown compound with those of reference samples recorded in a data base [*2*]. Using such a search window it has been shown that more than 99% of experimentally determined values fall within this range from the mean value [*3*].

In the present study, although the correlations obtained between RI data measured on capillary and packed columns are high (Table 3), several compounds gave RI values on capillary columns which differed by more than $\pm$50 units from those on packed columns and

TABLE 1—*GC retention indices on nonpolar bonded phase capillary columns and SE-30 packed columns,[a] in ascending order of retention index.[b]*

Compound	Retention Index			
	Narrow Bore 0.22-mm ID BP1 (SGE)	Wide Bore 0.53-mm ID BP1 (SGE)	Wide Bore 0.53-mm ID DB1 (J & W)	Packed Column[a]
Limonene[c]	1066	...[d]	...	1053
Amphetamine	1119	1122	...	1123
Phentermine	1155	1174	...	1148
Methylamphetamine	1177	1174	1174	1176
Ethosuximide	1196	1210	1190	1209
Nicotinic acid	1196(1388)	1158(1296,1343,1436)	1340(1290)	1335
Chlormethiazole	1221	1228	1221	1230
Mephentermine	1247	1233	1249	1239
Acetylsalicylic acid	1254(1446,1507)[e]	1252(1443,1333,1364)	1270(1450,1508)	1309
Salicylic acid	1277[e]	1296[e]	1266	1308
Warfarin	1325[e]	...	1326[e]	1432
Nicotine	1328	1327	...	1348
Phenelzine	1328	1329	...	1335
Salicylamide	1402	1422[e]	1397	1455
Phenmetrazine	1416	1425	...	1431
Barbitone	1461	1478[e]	1458	1497
Cantharidin	1472	1457	1468	1492
Diethylpropion	1476	1489	...	1486
Tolazoline	1477[e](1330,1198)	1474[e]	1465	1598,1490,1510
Paracetamol	1632	1643	...	1687
Butobarbitone	1642	1649	...	1665
Phenacetin	1660	1666	...	1675
Mescaline	1674	1688	...	1688
Amylobarbitone	1695	1705	1694	1718
Pentobarbitone	1720	1732	...	1740
γ-Benzenehexachloride	1751	1756	...	1739
Pethidine	1756	1767	...	1751
Meprobamate	1758	1764	...	1796
Quinalbarbitone	1772	1777	...	1791
Caffeine	1797	1803	...	1810

TABLE 1—*(Continued).*

Compound	Retention Index			
	Narrow Bore 0.22-mm ID BP1 (SGE)	Wide Bore 0.53-mm ID BP1 (SGE)	Wide Bore 0.53-mm ID DB1 (J & W)	Packed Column[a]
Pheniramine	1810	1820	...	1804
Glutethimide	1837	1840	...	1836
Thiopentone	1850	1855	...	1859
Diphenhydramine	1865	1872	...	1873
Lignocaine	1875	1882	...	1870
Methylphenobarbitone	1883	1888	...	1891
Theophylline	1909	1923	1904	1999
Phenobarbitone	1932	1937	1931	1957
Cyclobarbitone	1947	1955	1947	1963
Atropine	1966(2171,2046[e])	1976(2180,2058[e])	2175(1973,2051)	2199,2048
Psilocin	1967	1929	...	1976
Psilocybin	1968	1838(1927)	...	2046
Methapyrilene	1981	1993	...	1981
Cyclizine	2020	2027	...	2020
Methaqualone	2125	2129	2125	2125
Propranolol	2133	2139[e]	2138[e]	2157
Methadone	2139	2150[e]	...	2148
Cocaine	2177	2184	...	2187
Dextropropoxyphene	2180(1704,1619)	2186(1714)	2183(1623,1707)	2188,1687
Amitriptyline	2182	2188	2194	2196
Butriptyline	2185	2193	...	2181
Physostigmine	2196(1814)	2203(1829[e])	2198(1817)	2190,1804

Primidone	2242	2266	2239	2247
Nortriptyline	2265	2287	...	2210
Pindolol	2273	2294	2268	2260
Imipramine	2279	2295	2262	2223
Pentazocine	2326(2352,2503)	2339	2330	2275
Phenytoin	2350	2365	...	2330
Phenylbutazone	2404	2413	...	2365
Codeine	2441	2460	...	2376
Dihydrocodeine	2446	2465	2442	2365
Diazepam	2475	2493	2464	2425
Morphine	2492	2506	...	2455
Chlorpromazine	2549	2565	2553	2486
Thebaine	2559(2544)	2558(2576)	2555	2517
Prazepam	2679	2694	...	2641
Trifluoperazine	2714	2729	...	2683
Flurazepam	2799	2814	...	2785
Chlordiazepoxide	2809(2530)	2825(2543)	...	2799,2530,2453
Clonazepam	2832	2856	...	2885
Hydroxyzine	2889[e]	2906	...	2849
Ibogaine	2895(2969)	2908	2915	2871
Cholesterol	3093	3110	3100	3086
Strychnine	3125	3160	3140	3119
Buclizine	3285	3290	3295	3286

[a]Data from Ardrey et al. [2].
[b]For compounds giving multiple peaks, minor peaks are shown in parentheses.
[c]Limonene gave multiple peaks, the peak well separated from the solvent is the one given.
[d]... indicates no analysis was carried out.
[e]Slight peak tailing.

TABLE 2—*GC retention indices on nonpolar bonded phase capillary columns and SE-30 packed columns,[a] listed in alphabetical order.[b]*

Compound	Retention Index			
	Narrow Bore 0.22-mm ID BP1 (SGE)	Wide Bore 0.53-mm ID BP1 (SGE)	Wide Bore 0.53-mm ID DB1 (J & W)	Packed Column[a]
Acetylsalicylic acid	1254(1446,1507)	1252(1443,1333,1364)	1270(1450,1508)	1309
Amitriptyline	2182	2188	2194	2196
Amphetamine	1119	1122	...[c]	1123
Amylobarbitone	1695	1705	1694	1718
Atropine	1966(2171,2046)	1976(2180,2058)	2175(1973,2051)	2199,2048
Barbitone	1461	1478	1458	1497
γ-Benzenehexachloride	1751	1756	...	1739
Buclizine	3285	3290	3295	3286
Butobarbitone	1642	1649	...	1665
Butriptyline	2185	2193	...	2181
Caffeine	1797	1803	...	1810
Cantharidin	1472	1457	1468	1492
Chlordiazepoxide	2809(2530)	2825(2543)	...	2799,2530,2453
Chlormethiazole	1221	1228	1221	1230
Chlorpromazine	2549	2565	2553	2486
Cholesterol	3093	3110	3100	3086
Clonazepam	2832	2856	...	2885
Cocaine	2177	2184	...	2187
Codeine	2441	2460	...	2376
Cyclizine	2020	2027	...	2020
Cyclobarbitone	1947	1955	1947	1963
Dextropropoxyphene	2180(1704,1619)	2186(1714)	2183(1623,1707)	2188,1687
Diazepam	2475	2493	2464	2425
Diethylpropion	1476	1489	...	1486
Dihydrocodeine	2446	2465	2442	2365
Diphenhydramine	1865	1872	...	1873
Ethosuximide	1196	1210	1190	1209
Flurazepam	2799	2814	...	2785
Glutethimide	1837	1840	...	1836
Hydroxyzine	2889	2906	...	2849

Ibogaine	2895(2969)	2908	2915	2871
Imipramine	2279	2295	2262	2223
Lignocaine	1875	1882	...	1870
Limonene	1066	...	...	1053
Mephentermine	1247	1233	1249	1239
Meprobamate	1758	1764	...	1796
Mescaline	1674	1688	...	1688
Methadone	2139	2150	...	2148
Methapyrilene	1981	1993	...	1981
Methaqualone	2125	2129	2125	2125
Methylamphetamine	1177	1174	1174	1176
Methylphenobarbitone	1883	1888	...	1891
Morphine	2492	2506	...	2455
Nicotine	1328	1327	...	1348
Nicotinic acid	1196(1388)	1158(1296,1343,1436)	1340(1290)	1335
Nortriptyline	2265	2287	...	2210
Paracetamol	1632	1643	...	1687
Pentazocine	2326(2352,2503)	2339	2330	2275
Pentobarbitone	1720	1732	...	1740
Pethidine	1756	1767	...	1751
Phenacetin	1660	1666	...	1675
Phenelzine	1328	1329	...	1335
Pheniramine	1810	1820	...	1804
Phenmetrazine	1416	1425	...	1431
Phenobarbitone	1932	1937	1931	1957
Phentermine	1155	1174	...	1148
Phenylbutazone	2404	2413	...	2365
Phenytoin	2350	2365	...	2330
Physostigmine	2196(1814)	2203(1829)	2198(1817)	2190,1804
Pindolol	2273	2294	2268	2260
Prazepam	2679	2694	...	2641
Primidone	2242	2266	2239	2247
Propranolol	2133	2139	2138	2157
Psilocin	1967	1929	...	1976
Psilocybin	1968	1838(1927)	...	2046
Quinalbarbitone	1772	1777	...	1791
Salicylamide	1402	1422	1397	1455
Salicylic acid	1277	1296	1266	1308

TABLE 2—*(Continued).*

Compound	Retention Index			
	Narrow Bore 0.22-mm ID BP1 (SGE)	Wide Bore 0.53-mm ID BP1 (SGE)	Wide Bore 0.53-mm ID DB1 (J & W)	Packed Column[a]
Strychnine	3125	3160	3140	3119
Thebaine	2559(2544)	2558(2576)	2555	2517
Theophylline	1909	1923	1904	1999
Thiopentone	1850	1855	...	1859
Tolazoline	1477(1330,1198)	1474	1465	1598,1490,1510
Trifluoperazine	2714	2729	...	2683
Warfarin	1325	...	1326	1432

[a]Data from Ardrey et al. [2].
[b]For compounds giving multiple peaks, minor peaks are shown in parentheses.
[c]... indicates no analysis was carried out.

TABLE 3—*Correlation coefficients between GC columns (*NB = *narrow bore and* WB = *wide bore).*

	Column		
Column	SGE, BP1 WB	J & W, DB1 WB	SE-30 Packed
SGE, BP1 NB	0.9994	0.9975	0.9966
SGE, BP1 WB	...	0.9967	0.9952
J & W, DB1 WB	...	...	0.9980

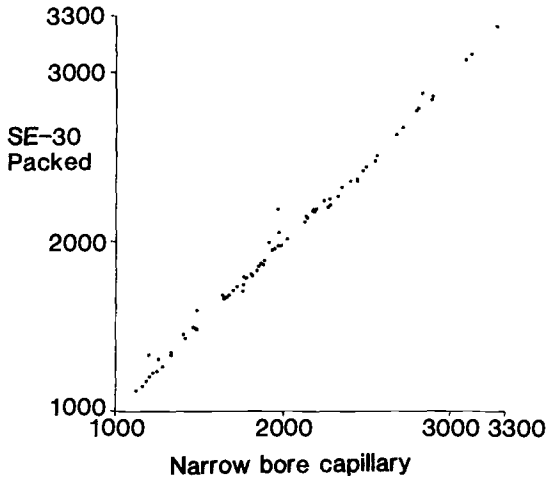

FIG. 1—*Correlation of the retention indices of drugs and other compounds of toxicological interest on narrow bore capillary and packed columns (*r = *0.9966).*

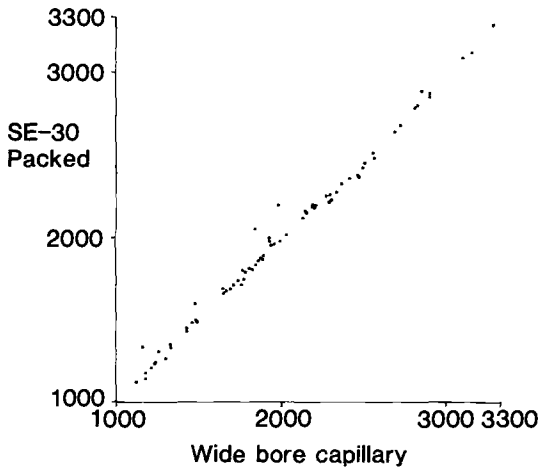

FIG. 2—*Correlation of the retention indices of drugs and other compounds of toxicological interest on wide bore capillary and packed columns (*r = *0.9952).*

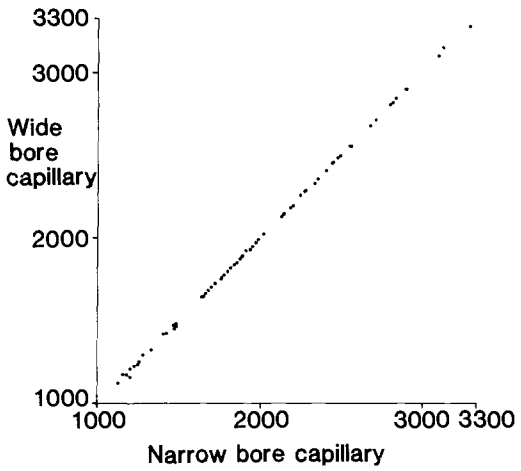

FIG. 3—*Correlation of the retention indices of drugs and other compounds of toxicological interest on SGE wide bore and narrow bore capillary columns (r = 0.9994).*

these compounds gave the outlying points on the correlation graphs in Figs. 1, 2, and 3. In addition, the elution orders of some pairs of closely eluting compounds (codeine, dihydrocodeine and amitriptyline, butriptyline) were reversed for packed and capillary columns. Decomposition of some drugs may also result in discrepancies between the observed RI and that recorded in a data base since experimental conditions may determine the degree of decomposition. This is illustrated by the analysis of psilocybin, when decomposition to psilocin can occur.

Compounds known to produce poor peak shapes on SE-30 packed columns did not chromatograph well on the capillary columns (nicotinic acid, acetylsalicylic acid, physostigmine, tolazoline, atropine, and warfarin), and several gave RI values which differed by more than ± 50 from the packed column data. The best peak shapes were obtained for some of these compounds on the DB1 wide bore capillary column. This may be attributable to the thicker film of stationary phase on this column (1.5 μm) since one of the claimed advantages of thick film columns is that residual "active sites" on the column wall are masked, thus reducing adsorption and tailing. During the period of the experimental evaluation of the wide bore columns, the DB1 column displayed less stationary phase bleed.

At present, there is not a data base available containing RI values of drugs generated solely on fused silica SE-30 bonded phase capillary columns which is as extensive as that generated on SE-30 packed columns and which contains interlaboratory data [*2*]. For the identification of unknown compounds eluting from capillary columns it would be very desirable to have access to such a data base containing RI values generated only on capillary columns since the present study shows that some discrepancies in RI values do occur between the two types of column. However, until such a data base is available, it is possible to use the packed column data for the preliminary identification of unknown compounds eluting from narrow bore and wide bore capillary columns providing that a wide enough search window is used. This generally presents few problems as unambiguous identification always requires confirmation by another technique (for example, mass spectrometry).

From this study, RI values of drugs determined on narrow bore capillary and wide bore capillary columns from two manufacturers were highly correlated indicating that a data base generated on narrow bore columns can be used for the identification of compounds eluting from wide bore columns or vice versa. Thus, if in the future, a data base, similar to that for

packed columns, is prepared for capillary columns, then this should be applicable to both narrow bore and wide bore columns. The comparison of drug RI values on the two different manufacturers' columns gave differences of up to 30 RI units for the two columns, well within the accepted search window used with packed columns. The correlations obtained for wide bore capillary columns were particularly interesting since these columns are becoming popular as alternatives to packed columns and discussions concerning their application to toxicological analyses are beginning to appear in the literature [*22,23*].

There has been much reported by other workers about the concentration dependence of retention behavior for capillary columns [*10,12,21,24–26*], especially at high loading. Newton and Foery [*12*] considered the influence of injecting different amounts of drug which might arise from samples containing therapeutic and toxic levels and found that most compounds only shifted from 1 to 8 RI units. For the present study, all RI values were determined using drug concentrations that did not overload the column. In practice, in a toxicology laboratory, any concentration related effects can be overcome since an overloaded peak is recognizable from its altered peak shape (slow upward rise with a rapid fall to baseline). Such a sample should be subsequently diluted and reinjected to redetermine its RI value. For wide bore columns, which have a larger sample capacity than narrow bore columns, the problem of overloading is diminished [*22*].

Conclusion

The isothermal retention indices of 75 compounds of toxicological interest measured on SE-30 equivalent narrow bore thin film capillary columns, wide bore thick film capillary columns, and packed columns are shown to be highly correlated. Retention indices on wide bore columns from different manufacturers are also highly correlated. The results indicate that it is reasonable to use RI data measured on SE-30 packed columns for the provisional assignment of identity of peaks eluting from wide bore or narrow bore capillary columns which have an SE-30-type stationary phase. As always in toxicological analyses, alternative methods must be used to confirm identity.

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