

# Comparison of Digital 12-Lead ECG and Digital 12-Lead Holter ECG Recordings in Healthy Male Subjects: Results from a Randomized, Double-Blinded, Placebo-Controlled Clinical Trial

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**Background:** Electrocardiogram (ECG) variability is greatly affected by the ECG recording method. This study aims to compare Holter and standard ECG recording methods in terms of central locations and variations of ECG data.

**Methods:** We used the ECG data from a double-blinded, placebo-controlled, randomized clinical trial and used a mixed model approach to assess the agreement between two methods in central locations and variations of eight ECG parameters (Heart Rate, PR, QRS, QT, RR, QTcB, QTcF, and QTcI intervals).

**Results:** A total of 34 healthy male subjects with mean age of  $25.7 \pm 4.78$  years were randomized to receive either active drug or placebo. Digital 12-lead ECG and digital 12-lead Holter ECG recordings were performed to assess ECG variability. There are no significant differences in least square mean between the Holter and the standard method for all ECG parameters except for QRS. The intraclass correlation coefficient (ICC) values for the Holter method are consistently lower than those for the standard method for all ECG parameters except for QRS, in particular, the ICC for QTcF is reduced from 0.86 for the standard method to 0.67 for the Holter method.

**Conclusions:** This study suggests that Holter ECGs recorded in a controlled environment are not significantly different but more variable than those from the standard method.

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TQT studies are currently the accepted method for investigating QTc interval changes of therapeutic and suprathreshold doses of new compounds.<sup>1</sup> However, Phase I studies often use doses up to the maximum tolerated dose (MTD) achieving plasma concentrations above those that will be seen during later stages of development, making single ascending dose and multiple ascending dose studies the ideal candidates for incorporation of early QT assessment. Traditionally ECG monitoring in these studies was very limited, primarily addressing

subject safety, and looking to exclude only large electrocardiographic abnormalities. Electrocardiogram (ECG) variability could be a result of trial design issues such as number and duration of ECGs, lack of ECG replicates and difficulty in controlling external environmental conditions such as stress. In addition, QT-RR hysteresis (the time lag of QT interval adaptation to heart rate changes) following changes in heart rate is an important factor in the variability of recorded ECG data and the outcome of QTc analysis.<sup>2–4</sup>

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The power of Phase I studies to detect QT interval changes can be improved by reduction of variability in the QT interval measurements. It has been speculated that quality QT data in single ascending dose and multiple ascending dose first in human studies can be collected without compromising the QT assessment by modifying the trial design features to integrate robust ECG monitoring and analyses.<sup>5-7</sup> ECG recordings should be performed at time points around  $C_{max}$  and the assessment should be conducted according to a controlled standardized procedure. Introduction of continuous Holter recordings has also become advantageous despite the common noise associated with the electrocardiographic signal. Furthermore, Holter ECG recordings can be available for retrospective analysis in a more intensive QT assessment in clinical trials and snapshots can be extracted according to the drug pharmacokinetic profile.

The use of an appropriate method for the measurement of QT and RR interval is therefore critical for the detection of small QTc effects in Phase I studies. The first study to compare the standard 12-lead ECG and digital 12-lead Holter recorders showed that these different methods provide equal value in the assessment of drug-induced changes from baseline in QT/QTc and RR intervals.<sup>8</sup> A meta-analysis study investigating the effects of (a) the baseline correction method (b) the design (c) the ECG recording method demonstrated that only the ECG recording method had a significant impact on data variability and that digitally recorded 12-lead Standard bedside ECG recordings were superior when compared to digital 12-lead Holter ECG recordings.<sup>9</sup>

In this study, the central level and variability of two commonly used digital ECG recording methods: 12-lead bedside ECG and 12-lead Holter ECG that were recorded simultaneously in the same subject, were compared and assessed using a mixed model approach.

## METHODS

This study used data collected from a Phase I, single-center, double-blinded, placebo-controlled, randomized, single-ascending oral dose and food interaction study to investigate the safety, tolerability and pharmacokinetics of ACT-280778 in healthy male subjects.<sup>10</sup> Each subject participated

in one treatment period only (fasted), with the exception of subjects in the food effect group who participated in two treatment periods (fed/fasted).

Tolerability and safety was evaluated by adverse events (AEs), physical examination, body weight, vital signs, ECG, and clinical laboratory tests. At various time-points before and after the administration of study drug, venous blood samples were collected for safety and pharmacokinetic analysis.

### ECG Assessment

Standard 12-lead paper ECG and Holter ECG were recorded with subjects at rest in the supine position for a 10-minute period (in triplicate) on Day -1, and on Day 1 at predose and at 1, 2, 4, 8, and 24 hours postdose. Bedside and 12-lead Holter recording methods were used simultaneously. Electrodes were placed for each system as close to each other as possible without interference. Subjects were connected to a 24-hour Getemed Holter ECG device (GE Healthcare, Chalfont St. Giles, United Kingdom) for simultaneous 12-lead ECG recording using a 12-lead Getemed Holter device digital recorder. The recorded data was used for retrospective 12-lead ECG snapshot extraction and analysis at the time-points specified in the clinical study protocol. In addition, standard 12-lead ECGs were recorded using a MAC1200/MAC1200ST ECG recorder (GE Healthcare) and stored electronically on the MUSE ECG Management System (GE Healthcare). Before any ECG recording, the subjects maintained an undisturbed supine resting position for at least 10 minutes and avoided postural changes during the ECG recordings. At each time point, the ECGs were recorded in triplicate, to reduce variance and improve the precision of measurement. Each ECG lasted 10 seconds. The triplicates were performed at 1-minute intervals during 3 minutes and the average of triplicates for each parameter was calculated and used for the statistical analyses. Mason-Likar ECG lead placement was used for both the standard recordings and Holter recordings. Dual ECG electrodes were utilized so that two leads can be attached to the same electrode. The Holter leads were connected to the top stud of the electrode, and the standard ECG leads to the bottom stud. In each case the ECG leads were connected via a 'crocodile clip'.

Prospective 12-lead ECG snapshot extraction from the 24-hour 12-lead Holter ECGs (GE Getemed) was done from within the 10-minute

rest period recording by a qualified cardiologist ensuring that the subject had had a minimum of 2 minutes of stable HR.

Automatic analysis was performed by the Marquette 12SL ECG Analysis Program (MEAP). All ECGs and their associated automated interval measurements were subsequently reviewed by qualified cardiologists. If manual adjustments of the automated measurement became necessary, a second cardiologist confirmed the assessment. Any disagreement between first and second reader was adjudicated by a third and most senior cardiologist. Details of this process have been described in.<sup>11</sup> This provided an opportunity to explore variability in the data which, owing to the simultaneous acquisition, are attributable to experimental noise.

### Statistical Analysis

Eight ECG parameters were analyzed: Heart Rate (bpm), PR interval (ms), QRS interval (ms), QT interval (ms), RR interval (ms), QTcB interval (ms), and QTcF interval (ms), and QTcI interval (ms) with particular emphasis on three corrected QTs: QTcF (Friderica), QTcB (Bazzett), and QTcI (Individual). The ECG variables QTcB (ms) and QTcF (ms) were calculated based on the QT and RR data retrieved from the standard 12-lead ECG and the 12-lead ECG obtained by snapshot extraction. QTcI was calculated using individual correction methods described in Wang et al.<sup>12</sup> To select the best correction method among six commonly used QT correction formulae, we used the least least-square regression method to choose the model with the minimum of the root mean squared error.<sup>12</sup>

The main purpose of the statistical analysis was to assess the agreement between Holter and standard ECG recording methods in central locations and variations of these ECG parameters. Arithmetic mean was used to describe the central level of an ECG parameter whereas standard deviation (SD) and range (maximum–minimum) were used to describe its variation. For inferential statistical analysis, a linear mixed effect model was used. Let  $y_{ijk}$  be an ECG parameter value of  $i$ th subject ( $i = 1, 2, \dots, n$ ) on Time  $k$  on  $j$ th Day. The mixed model can be expressed as:

$$y_{ijk} = \alpha + Method_i + Day_{jk} + Time_{ij} + S_i + \varepsilon_{ijk},$$

where  $\alpha$  is the intercept;  $Method_i$  is the fixed effect of method for  $i$ th subject, taking A and B for Holter and standard ECG recording method, respectively.  $Day_{jk}$  is the the fixed effect of the  $k$ th day, where  $j = 1, 2$  for Day -1 and 1, respectively;  $Time_{ij}$  is the fixed effect of the  $j$ th time point, where  $k = 1, 2, \dots, 6$  for predose, 1, 2, 4, 8, and 24 hours postdose, respectively;  $S_i$  is the between-subject effect of  $i$ th subject and  $\varepsilon_{ijk}$  is the the random error in observing  $y_{ijk}$ .

It is assumed that  $\{S_i\}$  are independently and identically distributed with mean 0 and variances  $\sigma_{sA}^2$  and  $\sigma_{sB}^2$ , and  $\{\varepsilon_{ijk}\}$  are independently distributed with mean 0 and variances  $\sigma_{eA}^2$  and  $\sigma_{eB}^2$ .  $\{S_i\}$  and  $\{\varepsilon_{ijk}\}$  are assumed mutually independent. The estimate of  $\sigma_{sA}^2$  and  $\sigma_{sB}^2$  are usually used to explain the inter-subject variability while  $\sigma_{eA}^2$  and  $\sigma_{eB}^2$  are used to assess the intrasubject variability for Holter and standard ECG recording method, respectively. The above model is sometimes called the heterogeneous variance mixed model.

Based on the above mixed model, the least square (LS) means of an ECG parameter for each ECG recording method, the differences between two methods together with 95% confidence interval were derived. In addition, inter-subject and intrasubject variability was estimated. The intersubject correlation coefficient (ICC) from the heterogeneous mixed model was also calculated. The ICC is an index of the reliability of the measurement for ECG recording method: it can be reasoned that the larger the ICC, the more reliable the method. The mixed regression model was estimated using SAS PROC MIXED.

### RESULTS

A total of 34 healthy male subjects were included in the study and were randomized to receive either ACT-280778 or matching placebo in five treatment periods (2 mg, 5 mg, 15 mg, 40 mg, and fed/fasted condition with 5 mg or matching placebo). Overall, subjects had a mean age of  $25.7 \pm 4.78$  years and a mean body mass index of  $24.6 \pm 2.28$  kg/m<sup>2</sup>. Of the 34 subjects included, 26 were Caucasian, 3 were Asian and 5 were black/African American. No serious adverse events were reported throughout the study and the novel nondihydropyridine dual L/T-type calcium channel blocker ACT-280778 was shown to be well tolerated.<sup>10</sup> Those

subjects contributed with 413 ECGs recorded by Holter method and 406 ECGs by standard method.

The descriptive statistics of the 8 ECG parameters by ECG recording method are presented in Table 1. These results indicate that no major differences in 8 ECG parameters between Holter and standard ECG recording methods were detected. The mean values for standard ECGs showed slightly higher PR, RR, QT, and QTcF. When the standard deviations (SD) from two methods are compared, some differences were observed (Table 1). HR and PR showed more variability when recorded using Holter ECG compared to standard ECG. QRS was the only parameter showing greater SD value for the standard ECG. SDs for QT, QTcB, and QTcF Holter recordings showed up to 3 ms greater variability compared to standard ECG.

Table 2 displays the mixed model analysis results of differences in LS mean ECG parameters between Holter and the standard method. There is no evidence to suggest that two methods produce different ECG readings in terms of LS mean values. For example, the difference in LS mean for QTcF between the Holter and the standard method is  $-0.69$ , 95%CI =  $-6.69, 5.31$ , P-value =  $0.8193$  and the difference of  $-26.51$  ms (95%CI:  $-70.24, 17.22$ , P value =  $0.2305$ ) in LS mean RR was due to chance.

From the mixed model analysis results of variations in ECG parameters between Holter and the standard method, four observations of relevance can be pointed out (Table 3). First, the total variance is consistently higher for Holter method than the standard method for all ECG parameters except for QRS. For example, Holter method results in over 50% increase in total variance for Heart Rate and QTcB. The variance for QTcF derived from the Holter traces is 25% higher in comparison to the standard method. Second, the intraclass correlation coefficient (ICC) values for the Holter method are consistently lower than those for the standard method for all ECG parameters except for QRS. For example, the ICC for QTcF is reduced from 0.86 for the standard method to 0.67 for the Holter method, about 22% reduction. Third, the Holter method has the largest impact on heart rate in terms of total variance and ICC. Finally, ICC values are consistently higher for QTcI than the QT, QTcB, and QTcF for both Holter and the standard method.

## DISCUSSION

Integration of intensive cardiac assessments in early-phase trials and collection of quality ECGs is highly desirable to assess QT effects at supratherapeutic doses usually evaluated in Phase I studies.

Constant supervision and quality control is required to produce consistently high quality ECGs over a period of time and unfavorable signal-to-noise ratio is unwanted as Phase I studies normally include a lower number of subjects when compared with dedicated TQT studies. Phase I studies can use both 12-lead ECG and Holter recordings. Holter monitoring allows the capture of entire exposure period of the drugs being investigated while also allowing recording of temporary variations in rhythm. On the other hand the scheduled 12-lead ECGs offer the opportunity to capture an effect while the subject is at rest ensuring that no autonomic tone interference and background noise are present. The 12-lead ECG point of analysis can however be poorly aligned with the PK data shifting the time of interest, therefore missing an effect. The implementation of continuous Holter monitoring as backup provides supplementary data analysis in Phase I studies.

The highest possible quality of ECG recordings should be ensured to help reduce unnecessary over analysis. Therefore this study aimed to compare digital 12-lead ECG and digital 12-lead Holter ECG recordings in a Phase I study environment as ECG variability can affect the conduct and interpretation of Phase I trials. In this study, we did not find significant differences in least square mean between the Holter and the standard method for any of the 8 ECG parameters. Nevertheless, the results from this study indicate that standard deviations for QT, QTcB and QTcF were higher for the data sets derived from Holter ECG traces except for QRS. Heart Rate and QTcB presented a total variance of over 50% using the Holter. This demonstrates that digitally recorded standard (12-lead bedside) ECGs are more accurate than digitally recorded Holter (12-lead) ECGs when using 10 second extraction with manual adjudication and that this effect is a combination of noise introduced during recording as well as at the extraction stage given that the over-reading follows the exact same process.

It was noted that the differences in mean value between the two methods (Holter and

**Table 1.** Descriptive Statistics of ECG Parameters by Standard and Holter Method

Parameter	Statistics	Holter	Standard
PR (ms)	N	411	406
	Mean (SD)	152.6(18.5)	155.1(17.0)
	Minimum-Maximum	106.0–196.0	120.0–194.0
Heart rate (bpm)	N	413	406
	Mean (SD)	60.1(7.9)	58.2(6.5)
	Minimum-Maximum	43.0–103.0	45.0–81.0
RR (ms)	N	413	406
	Mean (SD)	1013.9(123.7)	1043.2(111.5)
	Minimum-Maximum	583.0–1395.0	740.7–1333.3
QRS (ms)	N	413	406
	Mean (SD)	97.3(7.4)	96.5(8.5)
	Minimum-Maximum	76.0–118.0	72.0–118.0
QT (ms)	N	413	406
	Mean (SD)	398.4(23.8)	403.4(22.8)
	Minimum-Maximum	333.0–479.0	340.0–470.0
QTcB (ms)	N	413	406
	Mean (SD)	396.8(15.7)	395.7(12.8)
	Minimum-Maximum	349.6–451.4	357.8–430.7
QTcF (ms)	N	413	406
	Mean (SD)	397.2(14.7)	398.2(13.3)
	Minimum-Maximum	356.9–442.0	369.5–438.3
QTcI (ms)	N	413	406
	Mean (SD)	397.8(14.3)	397.1(12.1)
	Minimum-Maximum	360.9–443.4	367.2–433.4

**Table 2.** Comparison of Central Level of ECG Parameters between Standard and Holter Method: Mixed Model Analysis

Parameter	LS Means		Difference	95%CI		Probability
	Holter	Standard		Lower Limit	Upper Limit	
PR (ms)	152.11	154.00	– 1.89	– 9.99	6.22	0.6437
Heart Rate (bpm)	59.43	57.70	1.73	– 0.87	4.32	0.1887
RR (ms)	1025.10	1051.61	– 26.51	– 70.24	17.22	0.2305
QRS (ms)	97.50	96.82	0.68	– 2.66	4.01	0.6869
QT (ms)	400.80	404.95	– 4.15	– 13.85	5.55	0.3963
QTcB (ms)	396.81	395.66	1.15	– 4.41	6.71	0.6817
QTcF (ms)	397.98	398.67	– 0.69	– 6.69	5.31	0.8193
QTcI (ms)	398.95	397.70	1.24	– 4.40	6.88	0.6611

Mixed model includes day, time and method (Holter and Standard) as fixed effects and subject as random effect.

Standard) observed in this study were smaller than those observed in literature which showed QTcF differences in SD of up to 5–10 ms.<sup>9</sup> This is due to the fact that the recordings were made in the same subjects who were enrolled in the same study. The same ECG chest leads, same cardiologists and method of over-reading were used. Under these conditions, the Holter ECG data is improved compared to reported data, however, the challenge remains of selecting hysteresis free snapshots from Holter which even an experienced cardiologist may not always be able to do.

It was also observed in this study that the ICC values for the observed QT are higher than those for QTcB for both Holter and standard method although the total variances are still smaller for the QTcB. The Bazett's correction formula was derived based on a very small sample of 12 patients in 1920<sup>13</sup> and has been shown to overcorrect QT intervals at high heart rates and under-correct at low heart rates and therefore is not accurate method.<sup>14</sup> Fridericia's correction (QTcF) has the similar problems.<sup>14</sup> On the other hand, this study shows that among the uncorrected QT and the



**Table 3.** Comparison of Variability in ECG Parameters between Standard and Holter Method: Mixed Model Analysis<sup>a</sup>

Parameter	Holter				Standard				Holter/Standard	
	Variance				Variance				Total	
	Between-Subject	Within-Subject	Total	ICC <sup>b</sup>	Between-Subject	Within-Subject	Total	ICC	Variance	ICC
PR (ms)	288.74	66.82	355.56	0.81	262.70	28.86	291.56	0.90	1.22	0.90
Heart Rate (bpm)	24.97	36.70	61.67	0.40	28.08	12.45	40.53	0.69	1.52	0.58
RR (ms)	6926.54	8196.87	15123.41	0.46	8301.69	3748.60	12050.29	0.69	1.26	0.66
QRS (ms)	39.95	9.15	49.10	0.81	53.48	6.49	59.97	0.89	0.82	0.91
QT (ms)	379.31	170.71	550.02	0.69	399.74	91.48	491.22	0.81	1.12	0.85
QTcB (ms)	115.27	142.74	258.01	0.45	132.00	39.12	171.12	0.77	1.51	0.58
QTcF (ms)	146.47	73.48	219.95	0.67	151.60	25.01	176.61	0.86	1.25	0.78
QTcI (msec)	145.66	60.77	206.43	0.71	122.01	17.65	139.66	0.87	1.48	0.81

<sup>a</sup>Mixed model includes day, time and method (Holter and Standard) as fixed effects and subject as random effect.

<sup>b</sup>ICC refers to the intersubject correlation coefficient and calculated by between-subject variation/total variance.

corrected QTc (QTcB, QTcF, and QTcI), QTcI produces the smallest total variance and the largest ICC for both Holter and standard method. This is because QTc intervals are calculated from the individual correction formulae that best fitted the individual nonlinear relationships between QT and RR in terms of the root mean squared error, resulting in the lowest total variability and highest intrasubject stability of all corrected QT intervals.

The advantage of using 12-lead Holter devices in clinical studies is that they provide a continuous data acquisition available for retrospective analyses which can be used for example in safety reviews or beat to beat analysis. On the other hand, they make precise ECG acquisition more difficult leading to an increased variability due to QT/RR hysteresis<sup>9,15</sup> which is unwanted noise. Automated measurement becomes a necessity to provide large enough sample sizes to overcome this experimental noise, although it is acknowledged that it is sometimes difficult in practice to determine the end of the T wave which still limits the acceptability of automated ECG measurements.

The QT-RR relationship is primarily influenced by heart rate (HR) with rapid changes in HR often leading to QT-RR hysteresis resulting in greater variability of QTc measurements.<sup>2,3,15</sup> In addition, the autonomic nervous system is also thought to influence QT-RR which changes in response to sympathetic and vagal tone.<sup>16</sup>

A limitation of this study was the relatively small set of ECGs analyzed. However, this limitation did not seem to affect the study findings as they are in close agreement with published data.

In this Phase I study, we have used the mixed model to assess the agreement between Holter and standard ECG recording methods in terms of central locations and variations of ECG parameters. Advantages of the mixed model approach over conventional methods<sup>17,18</sup> are that it assesses the differences in locations and variations of ECG parameters simultaneously and it adjusts for the impact of covariates on the ECG measurements. Therefore, in a Phase I study environment, we encourage the use of the mixed model for ECG data analysis.

## CONCLUSION

Holter recording of electrocardiographic signal is usually disturbed by noise added to measured useful signal due to worse contact skin-electrode, body movements and uncontrolled external variants. Results from this study suggest that Holter ECGs recorded in a controlled environment are no different but more variable than those from the standard method. Results also indicate that differences in mean Holter ECG parameters between the two methods are smaller than those reported in the literature. However there is still a greater noise resulting from the difficulty in choosing Holter ECG for manual adjudication.

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